Instructions for use

BKV ELITe MGB® Kit

reagents for DNA Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF RTS175PLD

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IVD

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yyyy)
22–R	Expansion of the use of the product in association with MyGenius PRO (ref. INT050) instrument with plasma matrix. Expansion of the use of the product in association with the ELITe InGenius® and ELITe BeGenius® instruments and whole blood collected in EDTA (EDTA-WB) matrix. Update of the Appendixes: details on freeze/thaw procedure.	05/10/2026
21–R	Correction of typo errors: Inter - Session Repeatability on ELITe BeGenius (Table 20) and Limit of Detection for Plasma samples and ELITe GALAXY (Table 38).	15/05/2026
20–R	Expansion of the use of the product in association with MyGenius PRO (ref. INT050) instrument with urine matrix. Update of the paragraph “Notice To Purchaser: Limited License”. Update to “Model 2 version 4.0.2” of Assay protocols for the instruments ELITe InGenius® (REF INT030) and ELITe BeGenius® (REF INT040).	26/02/2026
19–R	Update for compliance with the Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) requirements. Upgrade of the analytical and diagnostic performances in PERFORMANCE CHARACTERISTICS paragraph Update of the Intended use: • Validation of the products in association with ELITe InGenius (REF INT030) and ELITe BeGenius (REF INT040) instruments with plasma collected in EDTA and urine collected without preservatives matrices. • Validation of the products in association with plasma collected in EDTA matrix and the following instruments: ELITe GALAXY and ABI 7500 Fast Dx Real-Time PCR Instrument. **NOTE** Composition of the product remains unchanged New graphics and content setting of the IFU.	26/09/2024
18	Extended Use of the product in association with ELITe BeGenius®» instrument Update of PERFORMANCE CHARACTERISTICS: • Change in Limit of Detection (LoD) • Change in Linear measuring range • Addition of Repeatability • Addition of Reproducibility	22/10/2021
17	Addition of the reference to the new product “BKV – ELITe Positive Control RF” (ref. CTR175PLD-R). Expansion of Use of the product in association with the platform Roche cobas z 480 analyzer.	25/01/2021
16	Correction of CV% value reported in “Precision with plasma samples and ELITe InGenius (sample volume 1000 µl)” table	01/08/2019
15	Modification of LoD and LoQ values for plasma and urine in association with ELITe InGenius® instrument.	11/06/2019
14	Extension of use with the extraction kit ELITe InGenius® SP 1000	05/07/2018
13	Update of Performance Characteristics section (ULoQ).	27/04/2018

12	Update of Performance Characteristics section (LoD and Linearity)	22/12/ 2017
00 — 11	New product development and succeeding changes	-

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1 INTENDED USE

The product **BKV ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as quantitative nucleic acids Real-Time PCR assay for the detection and quantification of the **DNA of human Polyomavirus BK (BKV)** extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of plasma collected in EDTA, urine collected without preservatives and whole blood collected in EDTA.

The assay is also validated in association with the **MyGenius PRO®** (registration name ELIVERSE®) instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of plasma collected in EDTA and urine collected without preservatives.

The assay is also validated in association with the **ELITe GALAXY**, automatic extraction and PCR set-up system and **7500 Fast Dx Real-Time PCR Instrument**, Real-Time PCR platform, using human specimens of plasma collected in EDTA.

The product is intended for use as an aid in the diagnosis and monitoring of BKV infections in patients suspected of having or undergoing monitoring of BKV infections.

The results must be interpreted in combination with all relevant clinical observations and laboratory outcomes.

2 ASSAY PRINCIPLE

The assay is a quantitative Real-Time PCR detecting BKV DNA isolated from specimens and amplified using the assay reagent BKV Q - PCR Mix, that contains primers and probes with ELITe MGB® technology.

The ELITe MGB probes are activated when hybridize with the related PCR products. **ELITe InGenius**, **ELITe BeGenius** and **MyGenius PRO** instruments monitor fluorescence increase and calculate the threshold cycles (Ct) and the melting temperatures (Tm). The BKV quantity is calculated based on a stored calibration curve.

7500 Fast Dx Real-Time PCR Instrument measures and records the increase of fluorescence emission. The subsequent data processing allows the detection and quantification of BKV in the primary specimen.

In the ELITe MGB probes the fluorophores are quenched in the random-coiled, single-stranded state of probe. The fluorophores are active in the probe / amplicon duplex as the quencher is spatially separated from the fluorophore. Note the fluorophore is not cleaved during PCR and can be utilized for dissociation analysis and melting temperature calculation.

3 PRODUCT DESCRIPTION

The **BKV ELITe MGB Kit** provides the assay reagent **BKV Q-PCR Mix**, an optimized and stabilized PCR mixture that contains the specific primers and probes for:

- a region of the **Large T antigen** gene of BKV, detected in Channel **BKV**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher® and labelled by FAM dye.
- Internal Control, specific for the **promoter and 5' UTR region** of the **human beta-globin gene,** detected in Channel **IC**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher and labelled by AquaPhluor® 525 (AP525) dye.

The **BKV Q-PCR Mix** also contains buffer, magnesium chloride, triphosphate nucleotides, AP593 fluorophore, (used instead of ROX or Cy5) as passive reference to normalize the fluorescence, the enzyme Uracil N-glycosidase (UNG) to inactivate contamination by the amplification product and the “hot start” DNA polymerase enzyme. The product **BKV ELITe MGB Kit** contains sufficient reagents for **96 tests** on **ELITe InGenius**, **ELITe BeGenius** and **MyGenius PRO with 20** µL used per reaction.

The product **BKV ELITe MGB Kit** contains sufficient reagents for **100 tests on other systems,** with **20** µL used per reaction.

NOTE
A conversion factor is required to express the results of the quantitative analysis in international units of BKV, in accordance with the “1st WHO International Standard for BK virus DNA” (NIBSC code 14/212, United Kingdom), is required.

4 MATERIALS PROVIDED IN THE PRODUCT

Table 1

Component	Description	Quantity	Classification of hazards
BKV Q-PCR Mix ref. RTS175PLD	Mixture of reagents for Real-Time PCR in tube with NATURAL cap	**4 x 540 µL**	**-**

5 MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench centrifuge (~5,000 RPM).
- Bench microcentrifuge (~13,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (volume range: 0.5-1000 µL).
- 2.0 mL sterile screw capped tubes (Sarstedt, ref. 72.694.005).
- 0.5 mL sterile screw capped tubes (Sarstedt, ref. 72.730.005)
- Molecular biology grade water.

6 OTHER PRODUCTS REQUIRED

The reagents for the extraction of sample DNA, the extraction and inhibition internal control, the amplification positive and negative controls, the DNA standards and the consumables **are not** provided with this product.

For the extraction of nucleic acids, Real-Time PCR and result interpretation of samples, the following products are required:

Table 2

Instruments and softwares	Products and reagents
ELITe InGenius (ELITechGroup S.p.A., EG SpA ref. INT030) **ELITe InGenius Software** version 1.4.0 (or later) **BKV ELITe_STD** , Assay Protocol with parameters for Calibrators analysis **BKV ELITe _PC**, Assay Protocol with parameters for Positive Control analysis **BKV ELITe _NC**, Assay Protocol with parameters for Negative Control analysis **BKV ELITe_PL_200_100** Assay Protocol with parameters for plasma specimen analysis **BKV ELITe_U_200_100** Assay Protocols with parameters for urine specimen analysis **BKV ELITe_WB_200_100** Assay Protocols with parameters for whole blood specimen analysis	**BKV - ELITe Standard** (EG SpA, ref. STD175PLD) **BKV - ELITe Positive Control** (EG SpA, ref. CTR175PLD) **CPE - Internal Control** (EG SpA, ref. CTRCPE) **ELITe InGenius** and **ELITe BeGenius** Consumables (see ELITe InGenius and ELITe BeGenius Instruction for use)
ELITe BeGenius (EG SpA ref. INT040) **ELITe BeGenius Software** version 2.3.0 (or later) **BKV ELITe_Be_STD**, Assay Protocol with parameters for Calibrators analysis **BKV ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis **BKV ELITe_Be_NC**, Assay Protocol with parameters for Negative Control analysis **BKV ELITe_Be_PL_200_100**, Assay Protocol with parameters for plasma specimen analysis **BKV ELITe_Be_U_200_100**, Assay Protocol with parameters for urine specimen analysis **BKV ELITe_Be_WB_200_100** Assay Protocols with parameters for whole blood specimen analysis	
MyGenius PRO (EG SpA ref: INT050). **MyGenius PRO** Software version BB-04 (or later) **BKV ELITe_My_STD,** Assay Protocol with parameters for Calibrators analysis **BKV ELITe_My_PC,** Assay Protocol with parameters for Positive Control analysis **BKV ELITe_My_NC,** Assay Protocol with parameters for Negative Control analysis **BKV ELITe_My_U_IU_200_100,** Assay Protocol with parameters for urine specimen analysis and result in IU/mL **BKV ELITe_My_U_cmL_200_100,** Assay Protocol with parameters for urine specimen analysis and result in copies/mL **BKV ELITe_My_PL_IU_200_100,** Assay Protocol with parameters for plasma specimen analysis and result in IU/mL **BKV ELITe_My_PL_cmL_200_100,** Assay Protocol with parameters for plasma specimen analysis and result in copies/mL	**BKV - ELITe Standard** (EG SpA, ref. STD175PLD) **BKV - ELITe Positive Control** (EG SpA, ref. CTR175PLD) **Negative Control** (EG SpA, ref. CTRNEG) **Internal Control Maxi** (EG SpA, ref. ICMAXI) **MyGenius PRO** Consumables (see MyGenius PRO Instruction for use)
ABI 7500 Fast Dx Real-Time PCR Instrument (ThermoFisher Scientific, ref. 4406985) **ELITe GALAXY** (EG SpA, ref. INT020) with **software version 1.3.1** (or later). Extraction Protocol for ELITe GALAXY, xNA Extraction (Universal)	**BKV - ELITe Standard** (EG SpA, ref. STD175PLD) **BKV - ELITe Positive Control** (EG SpA, ref. CTR175PLD) **CPE - Internal Control** (EG SpA, ref. CTRCPE) **ELITe GALAXY** Consumables (see ELITe GALAXY Instruction for use) **MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL** (Life Technologies, ref. 4346906), microplates with 0.1 mL wells and adhesive sealing sheets for real time amplification.

7 WARNINGS AND PRECAUTIONS

This product is designed for in-vitro use only.

7.1 General warnings and precautions

- Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with biological samples. Avoid splashing or spraying. Tubes, tips and other materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite (bleach) or autoclaved for one hour at 121°C before disposal.
- Handle and dispose of all reagents and all materials used to carry out the assay as if they were infectious. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal. Do not allow extraction reagents to contact sodium hypochlorite (bleach).
- Wear suitable protective clothes and gloves and protect eyes and face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Carefully wash hands after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with the regulations in force.
- Carefully read all the instructions provided before running the assay.
- While running the assay, follow the product instructions provided.
- Do not use the product after the indicated expiry date.
- Only use the reagents provided with the product and those recommended by the manufacturer.
- Do not use reagents from different batches.
- Do not use reagents from other manufacturers.

7.2 Warnings and precautions for molecular biology

Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to sample nucleic acid degradation or sample contamination by PCR products.

Never transfer lab coats, gloves or tools from the area designated for the amplification / detection of amplification products to the area designated for the extraction / preparation of the amplification reactions.

When the amplification session has to be performed with the 7500 Fast Dx Real-Time PCR instrument, it is necessary to have available separate areas for the extraction / preparation of amplification reactions and for the amplification / detection of amplification products. Never introduce an amplification product in the area designated for extraction / preparation of amplification reactions.

Laboratory coats, gloves and tools dedicated to work session setup are needed.

The samples must be suitable and, if possible, dedicated for this type of analysis. Samples must be handled under a laminar airflow hood. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, and free from DNA and RNA.

The reagents must be handled under a laminar airflow hood. The pipettes used to handle the reagents must be exclusively used for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, and free from DNA and RNA.

The extraction products must be handled to prevent dispersion into the environment and to avoid contamination of the instrument’s working area.

The PCR Cassette must be handled carefully and never opened to prevent PCR product diffusion and carryover contamination.

7.3 Warnings and precautions specific for the components

Table 3

Component	Storage temperature	Use from first opening	Free-ze / Thaw cy-cles	On board stability (ELITe InGenius and ELITe BeGenius)	On board stability (MyGenius PRO)
BKV Q-PCR Mix	-20 °C or below (protected from light)	60 days	up to five	up to five separate* sessions of three hours each or up to 7 consecutive hours (2 sessions of 3 hours each and the time needed to start a third session)	up to 7 consecutive hours or up to 3 consecutive hours for five times*

* with intermediate freezing.

8 SPECIMENS AND CONTROLS for ELITe InGenius, ELITe BeGenius AND MyGenius PRO

8.1 Specimens and Assay Protocols

This product is intended for use on **ELITe InGenius**, **ELITe BeGenius** and **MyGenius PRO** with the relative validated clinical specimens identified and handled according to laboratory guidelines, and collected, transported, and stored under the following conditions:

Table 4

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	+2 / +8 °C	-20 ± 10 °C	-70 ± 15 °C
Plasma	EDTA	≤ 1 d	≤ 3 d	≤ 30 d	≤ 30 d
Urine	without preservatives	≤ 4 hours	≤ 1d	≤ 30 d	≤ 30 d
Whole blood	EDTA	≤ 1 d	≤ 3 d	≤ 30 d	≤ 30 d

EDTA, Ethylenediaminetetraacetic acid; d, day.

Even if longer storage periods at -70 ° C are possible, as extensively reported by scientific literature, their application should be evaluated internally by the end-users of this product.

It is recommended to divide the specimens into aliquots before freezing to prevent repeated freeze/thaw cycles. When using frozen samples, thaw the samples just before the extraction to avoid possible nucleic acid degradation.

To perform samples testing on the **ELITe InGenius** and the **ELITe BeGenius**, the following Assay Protocols must be used. These IVD protocols were specifically validated with ELITe MGB Kits and the **ELITe InGenius** or **ELITe BeGenius** with the indicated matrices.

Table 5

Specimen	Instrument	Assay Protocol name	Report	Characteristics
Plasma in EDTA	ELITe InGenius	BKV ELITe_PL_200_100	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	BKV ELITe_Be_PL_200_100	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
Urine	ELITe InGenius	BKV ELITe_U_200_100	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	BKV ELITe_Be_UL_200_100	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
Whole blood	ELITe InGenius	BKV ELITe_WB_200_100	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	BKV ELITe_Be_WB_200_100	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

IU, international units

To perform samples testing on the **MyGenius PRO**, the following Assay Protocols must be used. These IVD protocols were specifically validated with ELITe MGB Kits and the **MyGenius PRO** with the indicated matrices.

Table 6 Assay protocols for BKV ELITe MGB Kit and MyGeniusPRO

Specimen	Instrument	Assay Protocol name	Report	Characteristics
Urine	MyGenius PRO	BKV ELITe_My_U_IU_200_100	IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
Urine	MyGenius PRO	BKV ELITe_My_U_cmL_200_100	copies/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
Plasma	MyGenius PRO	BKV ELITe_My_PL_IU_200_100	IU/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
Plasma	MyGenius PRO	BKV ELITe_My_PL_cmL_200_ 100	copies/mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

NOTE

Verify if the primary tube and the volume of the sample are compatible with ELITe InGenius, ELITe BeGenius or MyGenius PRO following the Instruction for use of the instruments.

The volume of the sample in a primary tube varies according to the type of tube loaded. Refer to the instructions for use of the extraction kit for more information on how to set up and perform the extraction procedure.

If required, 200 µL of sample must be transferred into an Extraction tube (for ELITe InGenius) or 2 mL Sarstedt Tube (for ELITe BeGenius), and 260 µL of sample into a 2 mL Sarstedt Tube (for MyGenius PRO).

NOTE

Pipetting samples to the **Extraction tube** or to the **2 mL Sarstedt Tube** might **generate contamination.** Use the appropriate pipettes and follow all recommendations reported in the “7 WARNINGS AND PRECAUTIONS page 8” section.

Purified nucleic acids can be left at room temperature for 16 hours and stored at -20 °C or below for no longer than one month.

Refer to “Potentially Interfering Substances” in the 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius, ELITe BeGenius and MyGenius PRO page 29 section to check data concerning interfering substances.

8.2 PCR calibrators and controls

The Calibration curve must be generated and approved for each lot of PCR reagent.

- For the calibration curve, use the four levels of the product **BKV ELITe Standard** (not provided with this kit) with the **BKV ELITe_STD**, **BKV ELITe_Be_STD** or **BKV ELITe_My_STD** Assay Protocols

NOTE

The concentration of Q – PCR Standards are expressed in copies / reaction (10^5 copies / rxn, 10^4 copies / rxn, 10^3 copies / rxn, 10^2 copies /rxn). Refer to “Standard Curve Uncertainty” in the 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius, ELITe BeGenius and MyGenius PRO page 29 section.

PCR control results must be generated and approved for each lot of PCR reagent.

- For the Positive Control, use the product **BKV - ELITe Positive Control** (not provided with this kit) with the **BKV ELITe_PC**, **BKV ELITe_Be_PC** or **BKV ELITe_My_PC** Assay Protocols
- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **BKV ELITe_NC**, **BKV ELITe_Be_NC** or use **Negative Control** (not provided with this kit) with the **BKV ELITe_My_NC** Assay Protocols

NOTE

The **ELITe InGenius**, **ELITe BeGenius** and **MyGenius PRO** allow generation and storage of the calibration curve and PCR control validation for each lot of PCR reagent.

NOTE

Calibration curves expire after **60 days**, at which time it is necessary to re-run the calibration.

PCR control results expire after **15 days**, at which time it is necessary to re-run the positive and negative controls.

The Calibrators and PCR controls must be re-run if any of the following events occur:

- a new lot of reagents is used,
- results of quality control analysis (see following paragraph) are out of specification,
- any major maintenance or service is performed on the **ELITe InGenius**, **ELITe BeGenius** and **MyGenius PRO** instruments.

8.3 Quality controls

Verification of the extraction and PCR procedure is recommended. Archived samples or certified reference material may be used. External controls should be used in accordance with local, state, and federal accrediting organizations, as applicable.

9 ELITe InGenius PROCEDURE

The procedure to use the **BKV ELITe MGB Kit** with the **ELITe InGenius** consists of three steps:

Table 7

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only)
		C) Calibration run (PCR Only)
		D) Positive Control and Negative Control run (PCR Only)
STEP 3	Review and approval of results	1) Validation of Calibration curve
		2) Validation of Positive Control and Negative Control results
		3) Validation of sample results
		4) Sample result reporting

9.1 STEP 1 – Verification of the system readiness

Before starting the session:

- switch on the **ELITe InGenius** and log in "**CLOSED**" mode,
- in the "Calibration" menu on the Home page, verify the Calibrators (**Q - PCR Standard**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid Calibrators are available for the **PCR Mix** lot, perform calibration as described in the following sections,
- in the "Controls" menu on the Home page, verify that the PCR Controls (**Positive Control**, **Negative Control**) are approved and valid (Status) for the PCR Mix lot to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- select the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and use the Assay Protocols provided by EG SpA (see 8 SPECIMENS AND CONTROLS for ELITe InGenius, ELITe BeGenius AND MyGenius PRO page 9).

If the Assay Protocol of interest is not loaded in the system, contact your local ELITechGroup Customer Service.

9.2 STEP 2 – Session Setup

The **BKV ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. Calibration run (PCR Only),

D. Positive Control and Negative Control run (PCR Only).

All required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

NOTE

The **ELITe InGenius** can be connected to the "Laboratory Information System" (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests**. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE
Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

To set up one of the four types of run follow the steps below while referring to the GUI:

	A. Sample run (Extract + PCR)	**B. Eluted sample run (PCR Only)**
1	**Identify samples** and, if needed, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. If required, transfer 200 µL of sample in an Extraction tube previously labelled. **Thaw** the needed **CPE tubes** at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 12 extractions.	**Thaw** the **Elution tube** containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.
2	Select “**Perform Run**” from the “Home” screen.	Select “**Perform Run**” from the “Home” screen.
3	Ensure the “Extraction Input Volume” is 200 µL and the Extracted Elute Volume is 100 µL.	Ensure the “Extraction Input Volume” is 200 µL and the Extracted Elute Volume is 100 µL.
4	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.
5	**Select** the **Assay Protocol** in the “Assay” column (see “Specimens and Controls”).	Select the **Assay Protocol** in the “Assay” column (see “Specimens and Controls”).
6	Ensure the “Protocol” displayed is: “Extract + PCR”.	Select “PCR Only” in the “Protocol” column.
7	Select the sample loading position as “Primary tube” or “Extraction Tube” in the “Sample Position” column. Ensure the “**Dilution factor**” is “**1**”.	Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Ensure the “**Dilution factor**” is “**1**”.
8	Click “Next” to continue.	Click “Next” to continue.
9	**Load CPE** and the **PCR Mix** on the “Inventory Block” referring to the “Load List” and enter CPE and PCR Mix lot number, expiry date and number of reactions for each tube.	**Load** the **PCR Mix** on the “Inventory Block” referring to the “Load List” and enter PCR Mix lot number, expiry date and number of reactions for each tube.
10	Click “Next” to continue.	Click “Next” to continue.
11	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.
12	Click “Next” to continue.	Click “Next” to continue.
13	**Load** PCR Cassette, ELITe InGenius SP 200 extraction cartridges, and all required consumables and samples to be extracted.	**Load** PCR Cassette and Elution tubes with samples extracted.
14	Click “Next” to continue.	Click “Next” to continue.
15	Close the instrument door.	Close the instrument door.
16	Press “Start”.	Press “Start”.

	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
1	**Thaw** the needed **Q-PCR Standard tubes** (Cal1: Q-PCR Standard 10^2, Cal2: Q-PCR Standard 10^3, Cal3: Q-PCR Standard 10^4, Cal4: Q-PCR Standard 10^5) at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Control tubes** at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block. **Prepare** the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITe InGenius SP 200 Consumable Set.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
3	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.	Ensure "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 100 µL.
4	For the Q-PCR Standard, assign the "Track", **select** the **Assay Protocol** (see "Specimen and Controls") in the "Assay" column and enter the reagent lot number and expiry date.	**Select** the **Assay Protocol** in the "Assay" column (see "Specimens and Controls"). Enter the lot number and expiry date of the Positive Control and of the molecular biology grade water.
5	Ensure "PCR Only" is selected in the "Protocol" column.	Ensure "PCR Only" is selected in the "Protocol" column.
6	Ensure the sample loading position in "Sample Position" column is "Elution Tube (bottom row)".	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)".
7	**Load** the **PCR Mix** on the "Inventory Block" referring to the Load List and enter the PCR Mix lot number, expiry date and number of reactions for each tube.	**Load** the **PCR Mix** on the "Inventory Block" referring to the "Load List" and enter the PCR Mix lot number, expiry date and number of reactions for each tube.
8	Click "Next" to continue.	Click "Next" to continue.
9	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.
10	Click "Next" to continue.	Click "Next" to continue.
11	**Load** the PCR Cassette and the Q-PCR Standard tubes.	**Load** PCR Cassette, Positive Control and Negative Control.
12	Click "Next" to continue.	Click "Next" to continue.
13	Close the instrument door.	Close the instrument door.
14	Press "Start".	Press "Start".

When the session is finished, the **ELITe InGenius** allows users to view, approve, store the results, print and save the report.

NOTE

At the end of the run the remaining Extracted Sample in the **Elution tube** must be removed from the instrument, capped, identified and stored at -20 ± 10 °C for no longer than one month. Avoid spilling of the Extracted Sample.

NOTE

At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block for up to 7 hours (2 sessions of 3 hours each and the time needed to start a third session); mix gently and spin down the content for 5 seconds before starting the next session.

NOTE

At the end of the run the remaining **Q - PCR Standard** can be removed from the instrument, capped, and stored at -20 °C or below. Avoid spilling the Q - PCR Standard.

NOTE

The **Q - PCR Standard** can be used for 4 separate sessions of 2 hours each.

NOTE

At the end of the run the remaining **Positive Control** can be removed from the instrument, capped and stored at -20 °C or below. Avoid the spilling of the Positive Control. The remaining **Negative Control** must be discarded.

NOTE

The **Positive Control** can be used for 4 separate sessions of 3 hours each.

NOTE

At the end of the run the **PCR Cassette** and the other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

9.3 STEP 3 - Review and approval of results

The **ELITe InGenius** monitors target and internal control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen the results and the run information are shown. From this screen results can be approved, and reports printed or saved (“Sample Report” or “Track Report”). Refer to the instrument manual for more details.

NOTE

The **ELITe InGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe InGenius** generates results with the **BKV ELITe MGB Kit** through the following procedure:

1. Validation of Calibration curve,
2. Validation of Positive Control and Negative Control results,
3. Validation of sample results,
4. Sample result reporting.

9.3.1 Validation of Calibration curve

The **ELITe InGenius software** interprets the PCR results for the target of the Calibrator reactions with the **ELITe STD** Assay Protocol parameters. The resulting Ct versus concentration produces the Calibration curve.

The Calibration curves, specific for the PCR reagent lot, are recorded in the database (Calibration). They can be viewed and approved by “Administrator” or “Analyst” users, following the GUI instructions.

The Calibration curve expires **after 60 days**.

NOTE

If the Calibration curve does not meet the acceptance criteria, the “Failed” message is shown on the “Calibration” screen. In this case, the results cannot be approved and the Calibrator amplification reactions must be repeated. In addition, if samples were included in the run, these are not quantified and must also be repeated to generate quantitative results.

9.3.2 Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius software** interprets the PCR results for the target of the Positive Control and Negative Control reactions with the **ELITe_PC** and **ELITe_NC** Assay Protocols parameters. The resulting Ct values are converted to concentration and used to verify the system (reagents lot and instrument).

The Positive Control and Negative Control results, specific for the PCR reagent lot, are recorded in the database (Controls). They can be viewed and approved by “Administrator” or “Analyst” users following the GUI instructions.

The Positive Control and Negative Control results expire **after 15 days**.

The ELITe InGenius software processes the Positive Control and Negative Control results and generates Control Charts. The approval of the Positive Control is based on the evaluation of the obtained logarithmic quantity that should be within the expected logarithmic quantity range (PC Chart). This ensures the system performance is within the acceptance criteria. The second chart (L-J chart) is dedicated exclusively to monitoring the Positive Control trend over time. Refer to the instrument manual for more details.

NOTE

If the Positive Control or Negative Control result does not meet the acceptance criteria, the “Failed” message is shown on the “Controls” screen. In this case, the results cannot be approved, and the Positive Control or Negative Control runs must be repeated.

NOTE

If the Positive Control or Negative Control result is not valid and samples were included in the same run, the samples can be approved but their results are not validated. In this case, the failed Control(s) and samples must all be repeated.

9.3.3 Validation of Sample results

The **ELITe InGenius Software** interprets the PCR results for the target (Channel **BKV**) and the Internal Control (Channel **IC**) with the **BKV ELITe_PL_200_100**, **BKV ELITe_U_200_100** or **BKV ELITe_WB_200_100** Assay Protocol parameters. The resulting target Ct values are converted to concentration.

Results are shown in "Results Display" screen.

The sample results can be approved when the three conditions in the table below are true.

1) Calibration Curve	**Status**
BKV Q-PCR Standard	APPROVED
2) Positive Control	**Status**
BKV Positive Control	APPROVED
3) Negative Control	**Status**
BKV Negative Control	APPROVED

The sample results are automatically interpreted by the **ELITe InGenius Software** using Assay Protocol parameters.

The possible result messages are listed in the table below.

For each sample the system reports a combination of the following messages specifying if the pathogen DNAs are either detected or not detected.

Result of sample run	**Interpretation**
BKV:DNA Detected, quantity equal to XXX copies / mL or IU/mL	**BKV DNA was detected** in the sample within the assay measurement range, its concentration is shown.
BKV:DNA Detected, quantity below LLoQ copies / mL or IU/mL	**BKV DNA was detected** in the sample, its concentration is below the assay Lower Limit of Quantification

Result of sample run	Interpretation
BKV:DNA Detected, quantity beyond ULoQ copies / mL or IU/mL	**BKV DNA was detected** in the sample, its concentration is above the assay Upper Limit of Quantification
BKV:DNA Not Detected or below LoD copies / mL or IU/mL	**BKV DNA was not detected** in the sample**.** The sample is negative for BKV DNA, or its concentration is below the assay Limit of Detection.
Invalid-Retest Sample	**Not valid assay result,** caused by Internal Control failure (due to e.g., incorrect extraction or inhibitors carry-over). The test should be repeated.

Samples reported as “Invalid-Retest Sample”: in this case, the Internal Control DNA was not efficiently detected, which could be due to problems in sample collection, extraction or PCR steps (e. g. incorrect sampling, degradation or loss of DNA during the extraction, or inhibitors in the eluate), which may cause incorrect results.

If sufficient eluate volume remains, the eluate can be retested (as is or diluted) by an amplification run in “PCR Only” mode. If the second result is invalid, the sample must be retested starting from extraction of a new sample using “Extract + PCR” mode (see 18 TROUBLESHOOTING page 52).

Samples reported as “BKV:DNA Not Detected or below “LoD” copies/mL or IU/mL" are suitable for analysis but BKV was not detected. In this case the sample may be either negative for BKV DNA or the BKV DNA is present at a concentration below the Limit of Detection of the assay (see 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius, ELITe BeGenius and MyGenius PRO page 29).

BKV DNA positive samples at a concentration below the Limit of Detection (and Lower Limit of Quantification) of the assay, if detected, are reported as “BKV:DNA Detected, quantity below “LLoQ” copies/mL or IU/mL” (see 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius, ELITe BeGenius and MyGenius PRO page 29).

BKV DNA positive samples within the Linear Measuring Range are detected and are reported as “BKV: DNA Detected, quantity equal to “XXX” copies/mL or IU/mL” (see 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius, ELITe BeGenius and MyGenius PRO page 29).

BKV DNA positive samples that are above the Upper Limit of Quantification are reported as “BKV:DNA Detected, quantity beyond “ULoQ” copies/mL or IU/mL” (see 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius, ELITe BeGenius and MyGenius PRO page 29), and they are not suitable for quantification. If needed the sample may be diluted before extraction or PCR and retested to yield results within the Linear Measuring Range of the assay.

NOTE

The results obtained with this assay must be interpreted in combination with all relevant clinical observation and laboratory outcomes.

The sample results are stored in the database and, if valid, can be approved (Results Display) by “Administrator” or “Analyst” users, following the GUI instruction. From the “Results Display” window it is possible to print and save the Sample run results as “Sample Report” and “Track Report”.

9.3.4 Sample result reporting

The sample results are stored in the database and reports can be exported as “Sample Report” and “Track Report”.

The “Sample Report” shows the results details by selected sample (SID).

The “Track Report” shows the results details by selected Track.

The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

10 ELITe BeGenius PROCEDURE

The procedure to use the **BKV ELITe MGB Kit** with the **ELITe BeGenius** consists of three steps:

Table 8

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only)
		C) Calibration run (PCR Only)
		D) Positive Control and Negative Control run (PCR Only)
STEP 3	Review and approval of results	1) Validation of Calibration curve
		2) Validation of Positive Control and Negative Control results
		3) Validation of sample results
		4) Sample result reporting

10.1 STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe BeGenius** and login "**CLOSED**" mode,
- in the "Calibrations" menu on the Home page, verify the Calibrators (**Q - PCR Standard**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid Calibrators are available for the **PCR Mix** lot, perform calibration as described in the following sections,
- in the "Controls" menu on the Home page, verify the PCR Controls (**Positive Control, Negative Control**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- select the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and use the Assay Protocols provided by EG SpA (see 8 SPECIMENS AND CONTROLS for ELITe InGenius, ELITe BeGenius AND MyGenius PRO page 9).

If the Assay Protocol of interest is not loaded in the system, contact your local ELITechGroup Customer Service.

10.2 STEP 2 – Session Setup

The **BKV ELITe MGB Kit** can be used on the **ELITe BeGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. Calibration run (PCR Only),

D. Positive Control and Negative Control run (PCR Only).

All the required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

NOTE

The **ELITe BeGenius** can be connected to the "Laboratory Information System" (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests**. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

To set up one of the four types of run follow the steps below while referring to the GUI:

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. If required, transfer 200 µL of sample in a 2 mL Sarstedt tube previously labelled. **Thaw** the needed **CPE tubes** at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 12 extractions.	**Thaw** the **Elution tube** containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.
2	Select "**Perform Run**" from the "Home" screen.	Select "**Perform Run**" from the "Home" screen.
3	Remove all the Racks from the "Cooler Unit" and place them on the preparation table.	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) of the "Cooler Unit" and place them on the preparation table.
4	Select the "Run mode": "Extract + PCR".	Select the "Run mode": "PCR Only".
5	Load the samples into the "Sample Rack". (Note: when secondary tubes "2 mL Tubes" are loaded, use the blue adaptors for the "Sample Rack").	Load the samples into the "Elution Rack".
6	**Insert the "Sample Rack"** into the "Cooler Unit" starting from the "Lane 5" (L5). If needed, insert the "Sample ID" (SID) for each "Position" used. (If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually the "Sample ID").	**Insert the "Elution Rack"** into the "Cooler Unit" starting from "Lane 3" (L3) If needed, for each "Position" enter the "Sample ID", the "Sample matrix", the "Extraction kit" and the "Extracted eluate vol." (eluate volume).
7	Click "Next" to continue.	Click "Next" to continue.
8	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.	Not applicable.
9	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").
10	Click "Next" to continue.	Click "Next" to continue.
11	When more than 12 samples are processed, repeat the procedure from point 6.	When more than 12 samples are processed, repeat the procedure from point 6.
12	Load the "Elution tubes" into the "Elution Rack" (Elution tubes can be labelled with barcode to improve traceability).	Not applicable.
13	Insert the "Elution Rack" into the "Cooler Unit" starting from "Lane 3" (L3). When more than 12 samples are processed, repeat using "Lane 2" (L2).	Not applicable.
14	Click "Next" to continue.	Not applicable.
15	Load CPE and the PCR Mix into the "Reagent/Elution Rack".	Load the PCR Mix into "Reagent/Elution Rack".
16	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). If needed, for each PCR Mix and / or CPE enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). If needed, for each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
17	Click "Next" to continue.	Click "Next" to continue.
18	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
19	Click “Next” to continue.	Click “Next” to continue.
20	Load the “PCR Rack” with “PCR Cassette” in the Inventory Area.	Load the “PCR Rack” with “PCR Cassette” in the Inventory Area.
21	Click “Next” to continue.	Click “Next” to continue.
22	Load the “Extraction Rack” with the “ELITe InGenius SP 200” extraction cartridges and required extraction consumables.	Not applicable.
23	Close the instrument door.	Close the instrument door.
24	Press “Start”.	Press “Start”.

	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
1	**Thaw** the needed **Q-PCR Standard tubes** (Cal1: Q-PCR Standard 10^2, Cal2: Q-PCR Standard 10^3, Cal3: Q-PCR Standard 10^4, Cal4: Q-PCR Standard 10^5) at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Control tubes** at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block. **Prepare** the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITe InGenius SP 200 Consumable Set.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
3	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.
4	Select the "Run mode: PCR Only".	Select the "Run mode": "PCR Only".
5	**Load the Q-PCR** Standard tubes into the "Elution Rack".	**Load** the **Positive Control and Negative Control tubes** into the "Elution Rack".
6	**Insert** the **"Elution Rack"** into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	**Insert** the **"Elution Rack"** into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
7	Click "Next" to continue.	Click "Next" to continue.
8	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").
9	Click "Next" to continue.	Click "Next" to continue.
10	**Load** the **PCR Mix** into "Reagent/Elution Rack".	**Load** the **PCR Mix** into "Reagent/Elution Rack".
11	**Insert** the "**Reagent/Elution Rack**" into the "Cooler Unit" in "Lane 2" (L2) If needed, for each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2). If needed, for each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
12	Click "Next" to continue.	Click "Next" to continue.
13	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Rack(s) if necessary.
14	Click "Next" to continue.	Click "Next" to continue.
15	**Load** the "**PCR Rack**" with "**PCR Cassette**" in the Inventory Area.	**Load** the "**PCR Rack**" with "**PCR Cassette**" in the Inventory Area.
16	Click "Next" to continue.	Click "Next" to continue.
17	Close the instrument door.	Close the instrument door.
18	Press "Start".	Press "Start".

When the session is finished, the **ELITe BeGenius** allows users to view, approve, store the results, print and save the report.

NOTE

At the end of the run, the remaining Extracted Sample in the **Elution tube** must be removed from the instrument, capped, identified, and stored at -20 ± 10 °C for no longer than one month. Avoid the spilling of the Extracted Sample.

NOTE

At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block for up to 7 hours (2 sessions of 3 hours each and the time needed to start a third session); mix gently and spin down the content for 5 seconds before starting the next session.

NOTE

At the end of the run, the remaining **Q - PCR Standard** can be removed from the instrument, capped and stored at -20 °C or below. Avoid spilling the Q - PCR Standard.

NOTE

The **Q- PCR Standard** can be used for 4 separate sessions of 2 hours each.

NOTE

At the end of the run, the remaining **Positive Control** can be removed from the instrument, capped and stored at -20 °C or below. Avoid the spilling of the **Positive Control**. The remaining **Negative Control** must be discarded.

NOTE

The **Positive Control** can be used for 4 separate sessions of 3 hours each.

NOTE

At the end of the run, the **PCR Cassette** and the other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

10.3 STEP 3 - Review and approval of results

The **ELITe BeGenius** monitors target and internal control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen the results and the run information are shown. From this screen results can be approved, and reports printed or saved (“Sample Report” or “Track Report”). Refer to the instrument manual for more details.

NOTE

The **ELITe BeGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe BeGenius** generates the results with the **BKV ELITe MGB Kit** through the following procedure:

1. Validation of Calibration curve,
2. Validation of Positive Control and Negative Control results,
3. Validation of sample results,
4. Sample result reporting.

NOTE

Please, refer to the same paragraph of the **ELITe InGenius Procedure** for the details.

11 MyGenius PRO PROCEDURE

The procedure to use the **BKV ELITe MGB Kit** with the **MyGenius PRO** consists of three steps:

Table 9

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample testing session (Extract + PCR)
		B) Calibration testing session (PCR Only)
		C) Positive Control and Negative Control testing session (PCR Only)
STEP 3	Review and approval of results	1) Validation of Calibration curve
		2) Validation of Positive Control and Negative Control results
		3) Validation of sample results
		4) Sample result reporting

STEP 1 - Verification of the system readiness

Before starting the testing session:

- switch on MyGenius PRO and log in; the unit will start in STAND-BY mode.
- in the “Calibrations” menu on the Home Screen, verify the Calibrators (**Q - PCR Standard**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid Calibrators are available for the **PCR Mix** lot, perform calibration as described in the following sections,
- in the “Controls” menu on the Home Screen, verify the PCR Controls (**Positive Control, Negative Control**) are approved and valid (Status) for the **PCR Mix** lot to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- follow the instructions on the Graphical User Interface (GUI) for the session setup and use the Assay Protocols provided by EG SpA (see “Specimens and Controls”).

If the Assay Protocol of interest is not loaded in the system, contact your local ELITechGroup Customer Service.

STEP 2 – Workflow Setup on the system

The **BKV ELITe MGB Kit** can be used on the **MyGenius PRO** to perform:

A. Sample testing session (Extract + PCR),

B. Calibration testing session (PCR Only),

C. Positive Control and Negative Control testing session (PCR Only).

All the required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

Before to setup a testing session:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests**. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE
Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

To set up one of the three types of testing session follow the steps below while referring to the GUI:

	A. Sample testing session (Extract + PCR)	B. Calibration testing session (PCR Only)	C. Positive Control and Negative Control testing session (PCR Only)
1	Identify samples and, if necessary, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. If the primary tube is compatible with MyGenius PRO racks, insert tube with samples into the racks; if this is not possible **transfer 260 µL of sample into a previously labelled secondary tube (2mL).** **Thaw** the needed Internal Control Maxi **IC MAXI tubes** at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds. Each tube is sufficient for 72 tests.	**Thaw** the needed **Q-PCR Standard tubes** (Cal1: Q- PCR Standard 102, Cal2: Q-PCR Standard 103, Cal3: Q-PCR Standard 104, Cal4: Q-PCR Standard 105) at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Control tubes** at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block. Spin down the **Negative Control tubes** for 5 seconds.
2	If the instrument is connected to the LIS, the sample barcodes are automatically read and recognized by the MyGenius PRO when placed in the Sample Area. If the instrument is not connected to the LIS, manually assign the samples using the **ASSIGN TEST** or **ASSIGN SAMPLE** functions on the "Sample List" page of the GUI, before loading them into the instrument. These functions also allow assigning the correct test protocol to each sample.	Load the PCR Mix and the Q-PCR Standard tubes into the refrigerated Reagent Carousel.	Load the PCR Mix and the Positive and Negative Controls tubes into the refrigerated Reagent Carousel.
3	In **STAND-BY** mode, check that there is a sufficient quantity of consumables for completing the test and that solid and liquid waste levels are adequate for the instrument to operate. If needed, load the required consumables into the appropriate drawers and empty the waste box and liquid tank. For detailed loading procedures, refer to the instrument's user manual.	In **STAND-BY** mode, check that there is a sufficient quantity of consumables for completing the test and that solid and liquid waste levels are adequate for the instrument to operate. If needed, load the required consumables into the appropriate drawers and empty the waste box and liquid tank. For detailed loading procedures, refer to the instrument's user manual.	In **STAND-BY** mode, check that there is a sufficient quantity of consumables for completing the test and that solid and liquid waste levels are adequate for the instrument to operate. If needed, load the required consumables into the appropriate drawers and empty the waste box and liquid tank. For detailed loading procedures, refer to the instrument's user manual.
4	Press Start in Home page and wait for the instrument to go into status 'Preparation' and then 'Operation'.	Press Start in Home page and wait for the instrument to go into status 'Preparation' and then 'Operation'.	Press Start in Home page and wait for the instrument to go into status 'Preparation' and then 'Operation'.
5	Load the PCR Mix in the Refrigerated Reagent Carousel.	Select "Calibration" button from the "Home" screen	Select "Controls" button from the "Home" screen
6	Book the opening of the door of the Auto Sampler Area and Load the Samples and IC MAXI.	Select in the Calibration page the Q-PCR Standard tube loaded in step 2 and then press "Order".	Select in the Control page the Positive Control and Negative Control loaded in step 2 and then press "Order".
7	Close the Auto Sampler door and the testing session starts.		

At the end of the testing session, the remaining **Q - PCR Standard** can be removed from the instrument, capped and stored at -20 °C or below. Avoid spilling the Q - PCR Standard.

NOTE

At the end of the testing session the **PCR Mix** can be removed from the instrument, capped and stored at -20 ° C or below or can be kept on board in the Carousel reagent for up to 7 hours.

As soon as a result is obtained, the **MyGenius PRO** allows users to view, approve, store the results and save the final report.

NOTE

The **Q- PCR Standard** can be used for 4 calibrations leaving them on board the instrument for a maximum of 2 hours for each calibration.

NOTE

At the end of the testing session, the remaining **Positive Control** can be removed from the instrument, capped and stored at -20 °C or below. Avoid the spilling of the **Positive Control**. The remaining **Negative Control** must be discarded.

NOTE

The **Positive Control** can be used for 4 calibrations leaving them on board the instrument for a maximum of 3 hours for each calibration.

NOTE

When necessary or when the instrument requires it, remove from the drawer of the instrument the **PCR Cassette** and the other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

NOTE

MyGenius PRO can be connected to the “Laboratory Information System” (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

STEP 3 -Review and approval of results

The **MyGenius PRO** monitors target and internal control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

When the analysis of each sample is complete, the results can be viewed in the ‘Results’ screen, showing the results and information about the samples. From this screen it is possible approve the results and save reports. See the instrument manual for more details.

The **MyGenius PRO** generates the results with the **BKV ELITe MGB Kit** through the following procedure:

1. Validation of Calibration curve,
2. Validation of Positive Control and Negative Control results,
3. Validation of sample results,
4. Sample result reporting.

Validation of Calibration curve

The **MyGenius PRO software** interprets the PCR results for the target of the Calibrator reactions with the **BKV ELITe_My_STD** Assay Protocol parameters. The resulting Ct versus concentration produces the Calibration curve.

The Calibration curves, specific for the PCR reagent lot, are recorded in the database (Calibrations). They can be viewed and approved by the user, selecting the calibration of interest and “View chart” section.

The Calibration curve expires **after 60 days**.

NOTE

If the Calibration curve does not meet the acceptance criteria, the “Error” message is shown on the “Calibrations” screen. In this case, the results cannot be approved, and the Calibrator amplification reactions must be repeated. In addition, if samples were included in the testing session, these are not quantified: in this case, after calibration repetition, all results must be interpreted and approved (see section dedicated to samples results).

Validation of amplification Positive Control and Negative Control results

The **MyGenius PRO Software** interprets the PCR results for the target of the Positive Control and for the target and the Internal Control (Channel **IC**) of the Negative Control reactions with the **BKV ELITe_My_PC** and **BKV ELITe_My_NC** Assay Protocols parameters. The resulting Ct values are converted to concentration and used to verify the system (reagents lot and instrument).

The Positive Control and Negative Control results, specific for the PCR reagent lot, are recorded in the database (Controls). They can be viewed and approved by the user selecting the control of interest and “View chart” section.

The Positive Control and Negative Control results expire **after 15 days**.

The **MyGenius PRO Software** processes the Positive Control and Negative Control results and generates Control Charts. The results are analyzed by the software to ensure the system performances are within the acceptance criteria, shown in the Control Chart plots. Refer to the instrument manual for more details.

NOTE

If the Positive Control or Negative Control result does not meet the acceptance criteria, the “Error” message is displayed on the “Controls” screen. In this case, the results cannot be approved, and the Positive Control or Negative Control must be repeated.

NOTE

If the Positive Control or Negative Control result is not valid and samples were included in the same testing session, the samples cannot be approved. In this case, after controls repetition, all results must be interpretated and approved (see section dedicated to samples results).

Validation of Sample results

The **MyGenius PRO Software** interprets the PCR results for the target (Channel **BKV**) and the Internal Control (Channel **IC**) with the **BKV ELITe_My_U_IU_200_100 and BKV ELITe_My_U_cmL_200_100, BKV ELITe_My_PL_IU_200_100 and BKV ELITe_My_PL_cmL_200_100** Assay Protocol parameters. The resulting target Ct values are converted to concentration.

Results are shown in "Results" section.

The sample results can be approved when the three conditions in the table below are true.

1) Calibration Curve	**Status**
BKV Q-PCR Standard	APPROVED
2) Positive Control	**Status**
BKV Positive Control	APPROVED
3) Negative Control	**Status**
BKV Negative Control	APPROVED

The sample results are automatically interpreted by the **MyGenius PRO Software** using Assay Protocol parameters if results of related calibration and controls are approved. If valid calibrations and controls are not present and approved when sample results are obtained, samples cannot be interpreted and approved: in this case, calibrations and controls must be approved and then, each sample must be interpreted in the “sample list” section and approved in the “Results” section using dedicated buttons.

The possible result messages are listed in the table below.

For each sample the system reports a combination of the following messages specifying if the pathogen DNAs are either detected or not detected.

Result of sample run	Interpretation
BKV:DNA DETECTED, QUANTITY EQUAL TO XXX COPIES/ML OR IU/ML	**BKV DNA was detected** in the sample within the assay measurement range, its concentration is shown.
BKV:DNA DETECTED, QUANTITY BELOW “LLOQ” COPIES/ML OR IU/ML	**BKV DNA was detected** in the sample, its concentration is below the assay Lower Limit of Quantification
BKV:DNA DETECTED, QUANTITY BEYOND “ULOQ” COPIES/ML OR IU/ML	**BKV DNA was detected** in the sample, its concentration is above the assay Upper Limit of Quantification
BKV:DNA NOT DETECTED OR BELOW “LOD” COPIES/ML OR IU/ML	**BKV DNA was not detected** in the sample**.** The sample is negative for BKV DNA, or its concentration is below the assay Limit of Detection.
INVALID - RETEST SAMPLE	**Not valid assay result,** caused by Internal Control failure (due to e.g., incorrect extraction or inhibitors carry-over). The test should be repeated.

Samples reported as “INVALID - RETEST SAMPLE”: in this case, the Internal Control DNA was not efficiently detected, which could be due to problems in sample collection, extraction or PCR steps (e. g. incorrect sampling, degradation or loss of DNA during the extraction, or inhibitors in the eluate), which may cause incorrect results. In this case the sample must be retested starting from extraction of a new sample.

Samples reported as “BKV: DNA NOT DETECTED OR BELOW “LOD” COPIES/ML OR IU/ML" are suitable for analysis but BKV was not detected. In this case the sample may be either negative for BKV DNA or the BKV DNA is present at a concentration below the Limit of Detection of the assay (see 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius, ELITe BeGenius and MyGenius PRO page 29).

BKV DNA positive samples at a concentration below the Limit of Detection (and Lower Limit of Quantification) of the assay, if detected, are reported as “BKV:DNA DETECTED, QUANTITY BELOW “LLOQ” COPIES/ML OR IU/ML” (see 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius, ELITe BeGenius and MyGenius PRO page 29).

BKV DNA positive samples within the Linear Measuring Range are detected and are reported as “BKV: DNA DETECTED, QUANTITY EQUAL TO XXX COPIES/ML OR IU/ML” (see 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius, ELITe BeGenius and MyGenius PRO page 29).

BKV DNA positive samples that are above the Upper Limit of Quantification are reported as “BKV: DNA DETECTED, QUANTITY BEYOND “ULOQ” COPIES/ML OR IU/ML” (see 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius, ELITe BeGenius and MyGenius PRO page 29), and they are not suitable for quantification. If needed the sample may be diluted before extraction or PCR and retested to yield results within the Linear Measuring Range of the assay.

NOTE

The results obtained with this assay must be interpreted in combination with all relevant clinical observation and laboratory outcomes.

The sample results are stored in the database and, if valid, can be approved (Results Display) by “Administrator” or “Analyst” users, following the GUI instruction. From the “Results Display” window it is possible to print and save the Sample run results as “Sample Report” and “Track Report”.

Sample result reporting

The sample results are stored in the database and reports can be exported as “Summay Report” and “Details Report”.

The “Details Report” shows the detailed results for each selected sample (SID).

The “Summary Report” shows the interpretation results for all the selected samples (SID).

The "Details Report" and "Summary Report" can be printed and signed by authorized personnel.

12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius, ELITe BeGenius and MyGenius PRO

12.1 Limit of Detection (LoD)

The Limit of Detection (LoD) of the assay in association to Plasma EDTA, Urine and Whole blood EDTA matrices was determined on ELITe InGenius or ELITe BeGenius instruments by testing a panel of BKV negative matrix spiked with reference material of BKV (1st WHO International Standard for BK Virus DNA, NIBSC ref. 14/212, United Kingdom). Probit regression analysis was performed on the results, and the LoD estimated as the concentration corresponding to 95% probability of a positive call.

The results for both matrices are reported in the following tables.

Table 10 Limit of Detection with ELITe InGenius (IU / mL)

Matrix	LoD	95% confidence range	
		lower limit	upper limit
urine	142 IU / mL	110 IU / mL	222 IU / mL
plasma	215 IU / mL	168 IU / mL	319 IU / mL
whole blood	538 IU / mL	337 IU/mL	1128 IU/mL

The analytical sensitivity as copies / mL for each matrices is calculated by applying the specific conversion factor reported at paragraph 12.9 Conversion factor to International Units page 35

The analytical sensitivity as copies / mL is reported below.

Table 11 Limit of Detection with ELITe InGenius (copies / mL)

Matrix	LoD	95% confidence range	
		lower limit	upper limit
urine	89 copies / mL	69 copies / mL	139 copies / mL
plasma	165 copies / mL	129 copies / mL	245 copies / mL
whole blood	117 copies / mL	73 copies / mL	245 copies / mL

The calculated LoD value was verified for each matrix by testing on ELITe InGenius and ELITe BeGenius a pool of each matrix spiked with BKV certified reference material at the claimed concentration.

The results obtained confirmed the claimed concentration for the target of BKV ELITe MGB Kit on both ELITe InGenius and ELITe BeGenius for each matrix.

The LoD values calculated on ELITe InGenius and Urine and plasma matrices are valid also for MyGenius PRO.

12.2 Inclusivity: Efficiency of detection on different strain or isolates

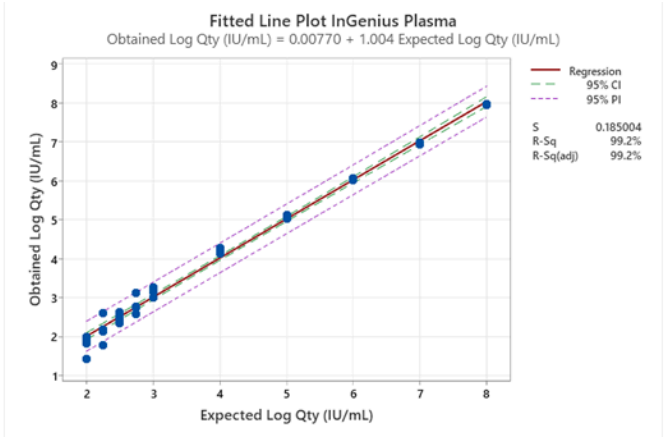
The Inclusivity of the assay, as efficiency of detection for different strain or isolates of BK Polyomavirus, was evaluated by in silico analysis. The analysis showed sequence conservation and absence of significant mutations. So, an efficient detection for the most of strains or isolates is expected.

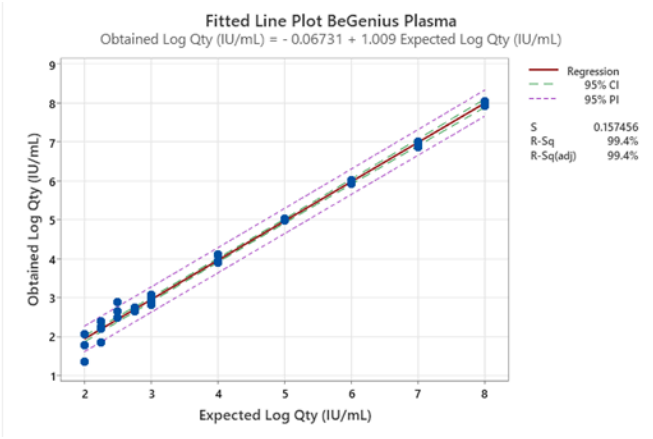
12.3 Linear measuring range and Limits of quantification

The linear measuring range of the assay was determined in association with Plasma EDTA, Urine and whole blood EDTA matrices on **ELITe InGenius** and **ELITe BeGenius** using a panel of dilution of BKV reference material (BKV Virus Culture Fluid, Heat inactivated, ZeptoMetrix) in BKV DNA - negative matrix.

The results for each matrix are reported in the following paragraphs.

Plasma:





The linear measuring range as copies / mL for Plasma EDTA is calculated by applying the specific conversion factor reported at paragraph 12.9 . page 35

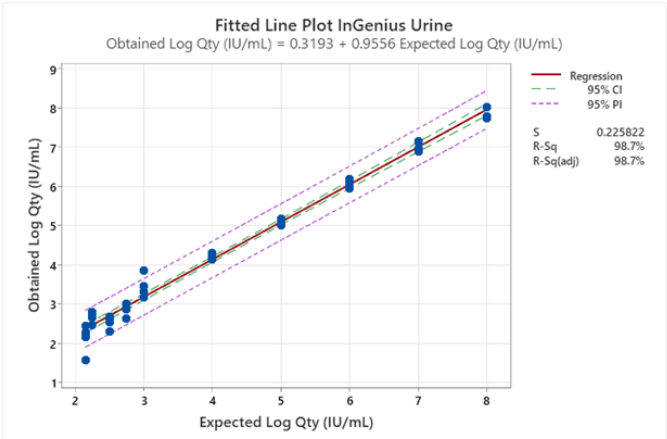
The final results are summarized in the following table.

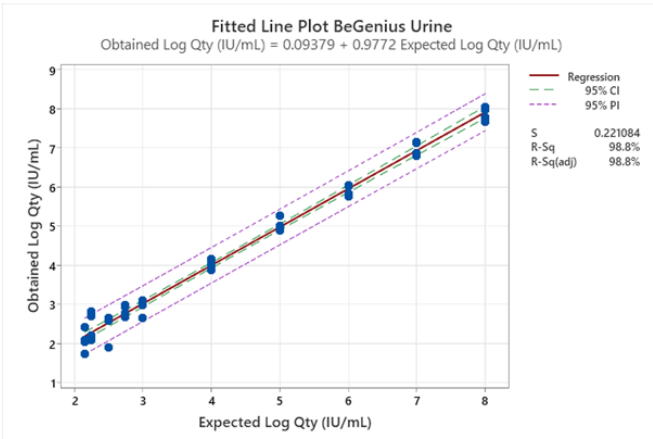
Table 12 Linear measuring range for plasma samples and ELITe InGenius and ELITe BeGenius

Unit	Lower limit	Upper limit
IU / mL	215	130,000,000
copies / mL	165	100,000,000

The Linear measuring range determined for ELITe InGenius and ELITe BeGenius and plasma matrix is valid also for MyGenius PRO.

Urine:





The linear measuring range as copies / mL for Urine is calculated by applying the specific conversion factor reported at paragraph 12.9 Conversion factor to International Units page 35

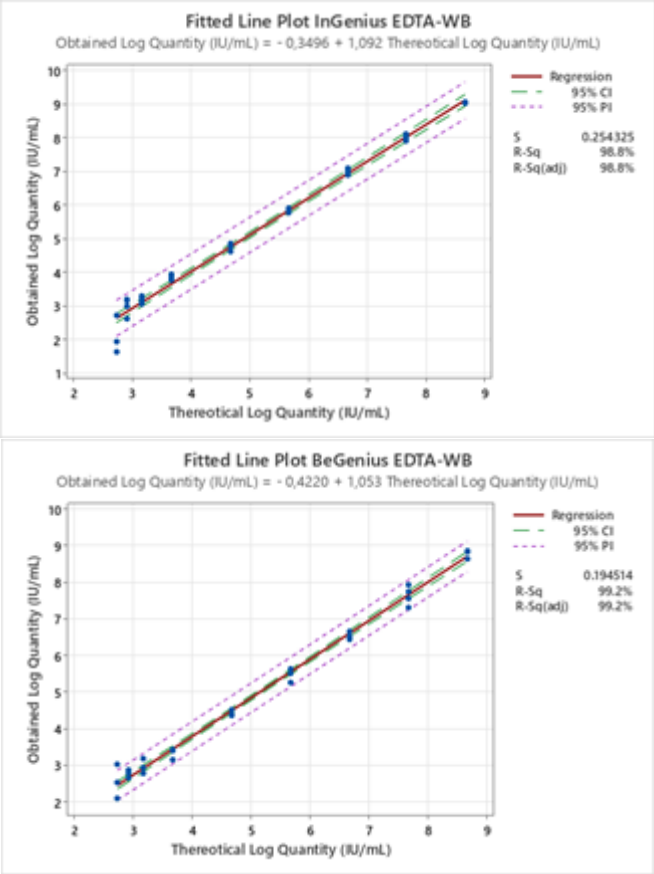
The final results are summarized in the following table.

Table 13 Linear measuring range for urine samples and ELITe InGenius and ELITe BeGenius

Unit	Lower limit	Upper limit
IU / mL	142	160,000,000
copies / mL	89	100,000,000

The Linear measuring range determined for ELITe InGenius and ELITe BeGenius and Urine matrix is valid also for MyGenius PRO.

Whole blood:



The linear measuring range as IU/ mL for EDTA-WB is calculated by applying the specific conversion factor reported at paragraph 12.9 Conversion factor to International Units page 35

The final results are summarized in the following table.

Table 14 Linear measuring range for EDTA-WB samples and ELITe InGenius and ELITe BeGenius

Unit	Lower limit	Upper limit
IU / mL	538	460,000,000
copies / mL	117	100,000,000

12.4 Standard Curve Uncertainty

The Uncertainty value of the Standard curve was calculated by combining the random errors (SD) of all level quantifications and multiplying for the Coverage factor k = 2 (Expanded Combined Uncertainty) and is equal to 0.2146 Log copies / reaction.

Table 15

Standard curve levels	Theoretical	Measured	Bias	SD	Expanded Combined Uncertainty
	Log c/rxn	Log c/rxn			
BKV Q - PCR Standard 10^5	5.0000	4.9845	0.0155	0.0417	0.2146
BKV Q - PCR Standard 10^4	4.0000	4.0022	-0.0022	0.0349	
BKV Q - PCR Standard 10^3	3.0000	3.0051	-0.0051	0.0500	
BKV Q - PCR Standard 10^2	2.0000	2.0471	-0.0471	0.0778	

12.5 Potential interfering organisms: Cross-reactivity

The potential cross-reactivity with other unintended organisms of the product BKV ELITe MGB Kit was evaluated by in silico analysis of sequences available in the EBI ENA nucleotide database. The analysis showed no significant sequence homology with other unintended organisms (viruses, bacteria and fungi). So, no cross-reactivity or interference is expected.

12.6 Potential interfering substances: Inhibition

The potential inhibition of interfering substances (endogenous and exogenous) that might be found in clinical specimens was evaluated for the assay by analysis of a panel of substances at relevant concentration in BKV positive samples.

The results, for each matrix, are reported in the following tables.

Table 16 Plasma

Substance	Pos. / Rep	Outcome
Azithromycin	5 / 5	No interference
Ganciclovir	5 / 5	No interference
Ribavirin	5 / 5	No interference
Abacavir	5 / 5	No interference
Cidofovir	5 / 5	No interference
Cyclosporine A	5 / 5	No interference
Bilirubin	5 / 5	No interference
EDTA	5 / 5	No interference
Heparin	5 / 5	No interference

The tested substances do not interfere with the BKV or Internal Control amplification.

Table 17 Urine

Substance	Pos. / Rep.	Outcome
Azithromycin	5 / 5	No interference
Bilirubin	5 / 5	No interference

Table 17 Urine (continued)

Substance	Pos. / Rep.	Outcome
Whole Blood	5 / 5	No interference
Phenazopyridine Hydrochloride	5 / 5	No interference

The tested substances do not interfere with the BKV or Internal Control amplification.

Table 18 Whole blood

Substance	Pos. / Rep.	Outcome
Haemolytic Blood High	5 / 5	No interference
Haemolytic Blood Medium	5 / 5	No interference
Haemolytic Blood Low	5 / 5	No interference
Triglycerides	5 / 5	No interference
Bilirubin	5 / 5	No interference
Heparin	5 / 5	No interference
Ganciclovir	5 / 5	No interference
Cidofovir	5 / 5	No interference
Ribavirin	5 / 5	No interference
Azithromycin	5 / 5	No interference
Vancomycin	5 / 5	No interference
Amoxicillin	5 / 5	No interference
Fluconazole	5 / 5	No interference
Hydrocortisone	5 / 5	No interference
Cyclosporine A	5 / 5	No interference

The tested substances do not interfere with the BKV or Internal Control amplification.

12.7 Repeatability

The Intra-Session and Inter-Session Repeatability of the assay was evaluated on ELITe InGenius and ELITe BeGenius by analysis of a panel of Plasma samples collected in EDTA, including one negative sample and two samples spiked by BKV certified reference material (1st WHO International Standard for BKV virus DNA" NIBSC code 14/212, United Kingdom).

An example of Intra-Session Repeatability (on one day) results is shown in the tables below.

Table 19 Intra – Session Repeatability on ELITe InGenius

Sample	BKV				
	N	Mean Ct	SD	% CV	% Agreement
Negative	8	-	-	-	100%
3 x LoD	8	36.66	0.45	0.82	100%
10 x LoD	8	34.88	0.56	1.33	100%

Table 20 Intra – Session Repeatability on ELITe BeGenius

Sample	BKV				
	N	Mean Ct	SD	% CV	% Agreement
Negative	8	-	-	-	100%
3 x LoD	8	37.09	0.52	1.40	100%
10 x LoD	8	35.45	0.31	0.88	100%

An example of Inter-Session Repeatability results is shown in the tables below.

Table 21 Inter – Session Repeatability on ELITe InGenius

Sample	BKV- Days 1-2				
	N	Mean Ct	SD Ct	% CV Ct	% Agreement
Negative	16	-	-	-	100%
3 x LoD	16	36.36	0.52	1.43	100%
10 x LoD	16	34.40	0.68	1.96	100%

Table 22 Inter – Session Repeatability on ELITe BeGenius

Sample	BKV - Days 1-2				
	N	Mean Ct	SD Ct	% CV Ct	% Agreement
Negative	16	-	-	-	100%
3 x LoD	16	36.68	0.71	1.94	100%
10 x LoD	16	34.98	0.55	1.57	100%

In the Repeatability test, the BKV ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values with %CV lower than 5%.

12.8 Reproducibility

The Reproducibility of the assay was evaluated on ELITe InGenius and ELITe BeGenius by analysis of a panel of Plasma samples collected in EDTA negative or spiked with BKV (1st WHO International Standard for BKV virus DNA" NIBSC code 14/212, United Kingdom).

A summary of Inter-Instrument Reproducibility (on two instruments) is shown in the tables below.

Table 23 Inter–Instrument Reproducibility on ELITe InGenius

Sample	BKV				
	N	Mean Ct	SD	% CV	% Agreement
Negative	8	-	-	-	100%
3 x LoD	8	36.72	0.30	0.82	100%
10 x LoD	8	30.89	0.41	1.33	100%

Table 24 Inter–Instrument Reproducibility on ELITe BeGenius

Sample	BKV				
	N	Mean Ct	SD	% CV	% Agreement
Negative	8	-	-	-	100%
3 x LoD	8	36.87	0.58	1.56	100%
10 x LoD	8	34.86	0.25	0.72	100%

A summary of Inter-batch Reproducibility (on two lots) is shown in the tables below:

Table 25 Inter–Batch Reproducibility on ELITe InGenius

Sample	BKV				
	N	Mean Ct	SD	% CV	% Agreement
Negative	8	-	-	-	100%
3 x LoD	8	36.94	0.36	0.82	100%
10 x LoD	8	35.07	0.28	1.33	100%

Table 26 Inter–Batch Reproducibility on ELITe BeGenius

Sample	BKV				
	N	Mean Ct	SD	% CV	% Agreement
Negative	8	-	-	-	100%
3 x LoD	8	36.81	0.66	1.56	100%
10 x LoD	8	35.01	0.41	0.72	100%

In the Reproducibility test, the BKV ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values with %CV lower than 5%.

12.9 Conversion factor to International Units

The conversion factor to report the quantitative results in International Units / mL starting from copies / mL, was calculated, for each matrix, using the certified calibrated reference material “1st WHO International Standard for BKV virus DNA” (NIBSC code 14/212, United Kingdom).

The results for each matrix are summarized in the following table

Table 27 Conversion factor to International Units

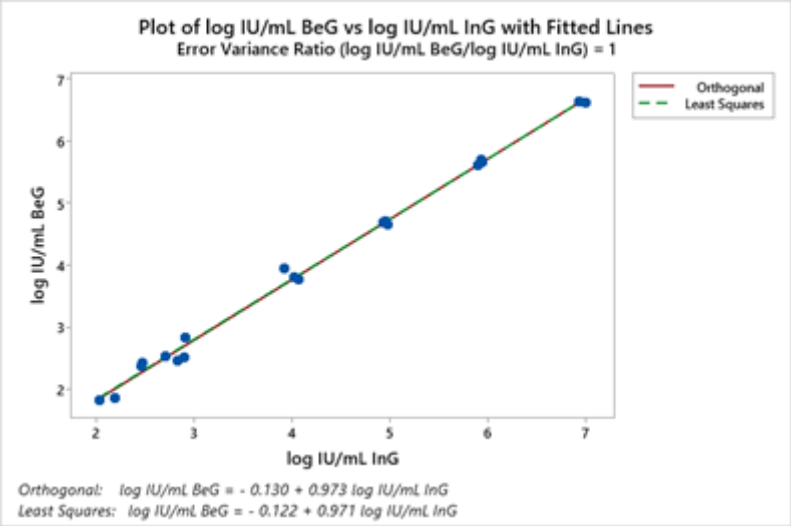
Matrix	Fc (IU / copies)
Plasma	1.3
Urine	1.6
Whole blood	4.6

The Conversion factors to International Units calculated for ELITe InGenius and Urine and plasma matrices are valid also for MyGenius PRO.

The Conversion factor, to report the quantitative results in International Units / mL starting from copies / mL, was verified for each matrix on **ELITe InGenius** and **ELITe BeGenius** instruments using the certified calibrated reference material (1st WHO International Standard, NIBSC). The results obtained were analysed by orthogonal and linear regression in order to calculate the correlation.

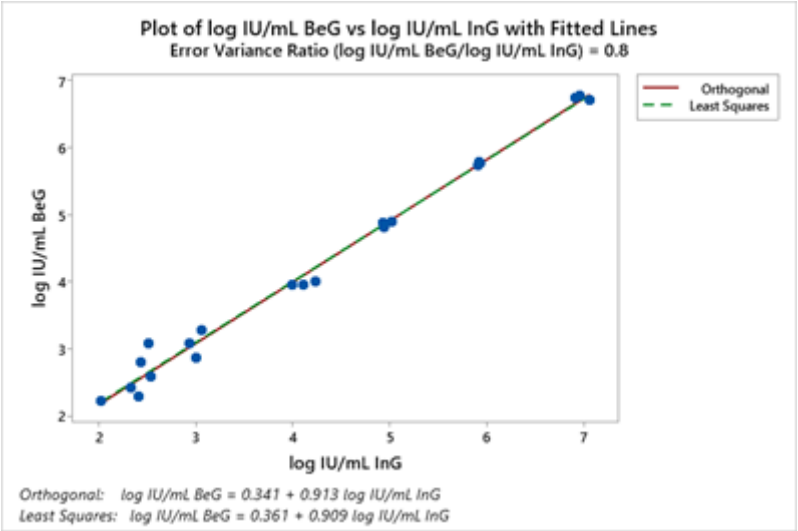
The results, for each matrix, are reported in the following paragraphs.

Plasma



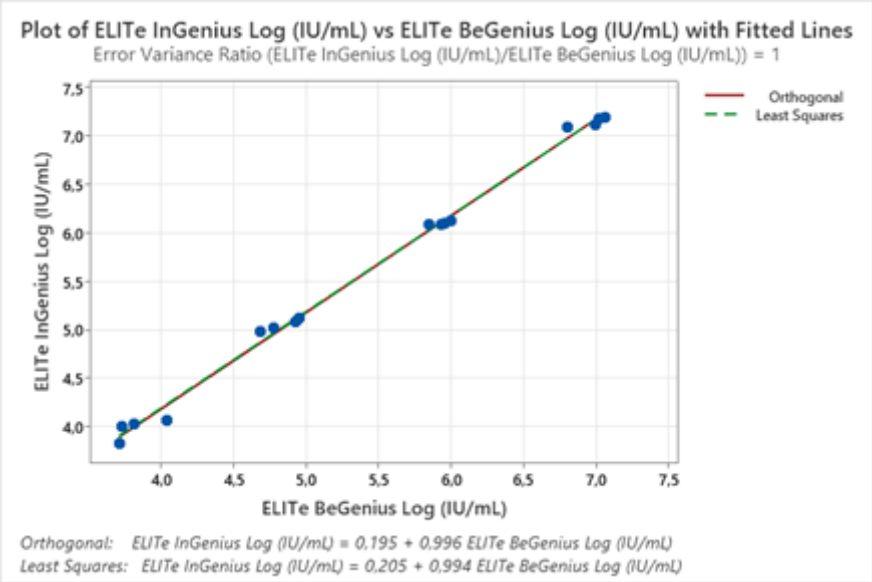
The Orthogonal Regression analysis generated an intercept equal to -0.130 (95% CI: - 0.263 – 0.002) and a slope equal to 0.973 (95% CI: 0.944 - 1.001).

Urine:



The Orthogonal Regression analysis generated an intercept equal to 0.341 (95% CI: 0.152 – 0.529) and a slope equal to 0.913 (95% IC: 0.872 - 0.954).

Whole blood:



The Orthogonal Regression analysis generated an intercept equal to 0.195 (95% CI: 0.0174; 0.3733) and a slope equal to 0.996 (95% CI: 0.9639; 1.0284).

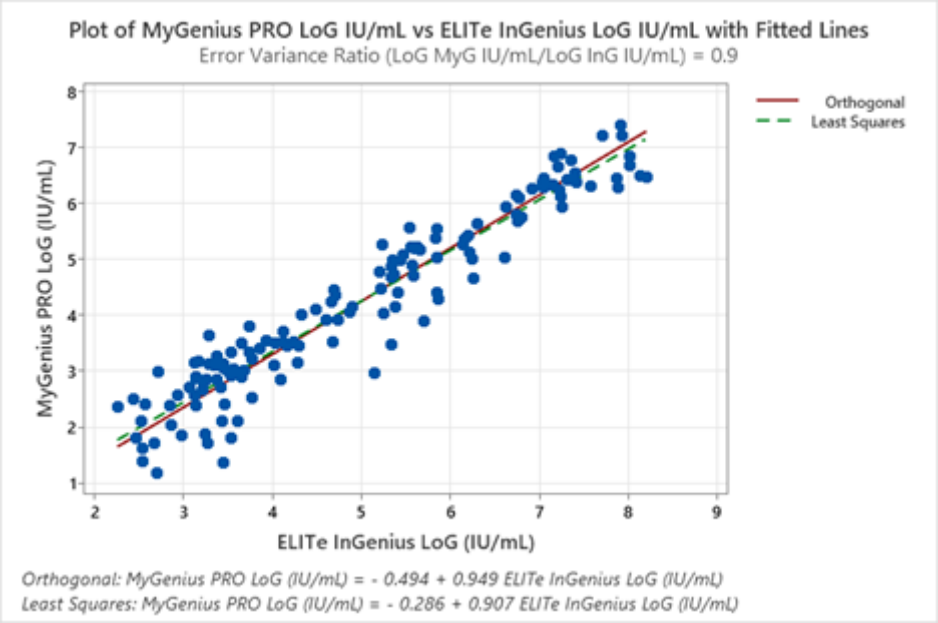
12.10 MyGenius PRO: method correlation

The correlation analysis of different methods was evaluated on MyGenius PRO by analysing BKV samples from patients whose viral load was within the measuring range of the reference methods (ELITe InGenius). The results obtained with the MyGenius PRO and the reference method (ELITe InGenius) were analysed by Deming and Linear Regression.

Urine

The correlation study was performed at one site on 137 positive clinical samples of urine certified positive for BKV DNA or spiked with reference material using the ELITe InGenius as comparator.

The results are summed up in the following figure.

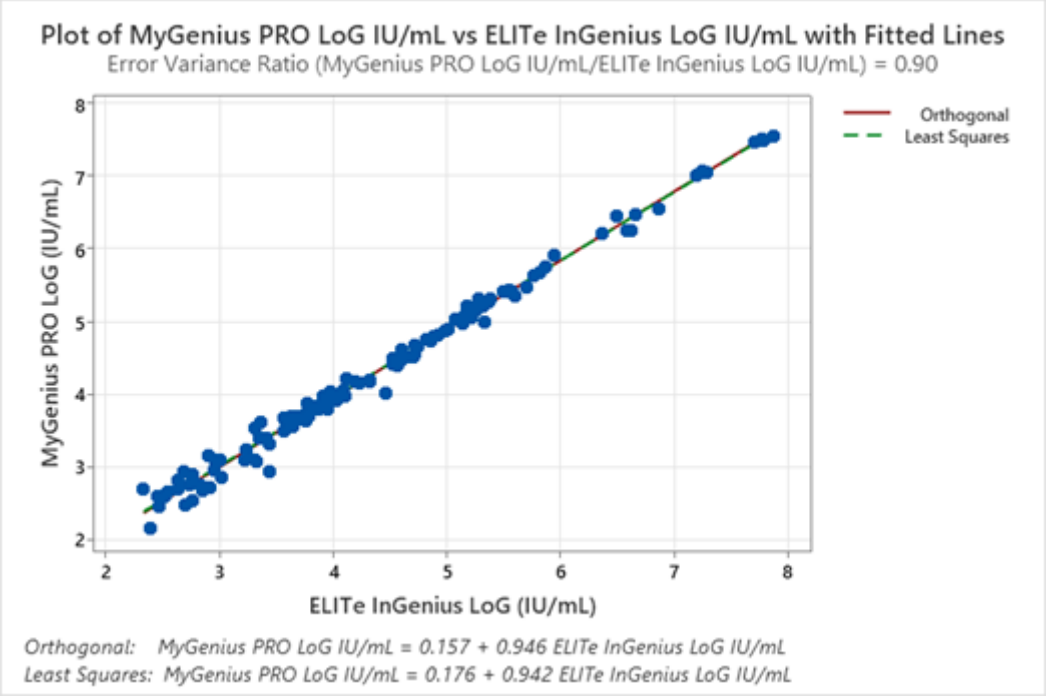


The Deming Regression analysis generated an intercept equal to -0.494 (95% CI -0.7502; -0.2371) and a slope equal to 0.949 (95% CI: 0.8996; 0.9982). The linear regression analysis generated an R2 of 0.913.

Plasma

The correlation study was performed at one site on 122 positive clinical samples of plasma certified positive for BKV DNA or spiked with reference material using the ELITe InGenius as comparator.

The results are summed up in the following figure.



The Deming Regression analysis generated an intercept equal to 0.1573 (95% CI: 0.0829; 0.2316) and a slope equal to 0.9464 (95% IC: 0.9304; 0.9624). The linear regression analysis generated an R2 of 0.991.

12.11 Diagnostic Specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative samples, was evaluated in association with **ELITe InGenius** or **ELITe BeGenius,** analysing clinical samples certified negative or presumably negative for BKV DNA. As **ELITe BeGenius** showed equivalent analytical performances to **ELITe InGenius**, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic Specificity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius and vice versa.

As **MyGenius PRO** showed equivalent analytical performances to ELITe InGenius in association to urine matrix and plasma matrix, the diagnostic performances of the assay performed on the two instruments and urine and plasma are also considered equivalent. Therefore, the Diagnostic Specificity of the assay obtained in association with ELITe InGenius is also applicable to MyGenius PRO.

The results are summed up in the following table.

Table 28 Diagnostic Specificity

Samples	N	Positive	Negative	% Diagnostic Specificity
Plasma collected in EDTA negative for BKV DNA	79	3	76	**96.2%**
Urine without preservatives negative for BKV DNA	68	0	68	**100%**
Whole blood collected in EDTA negative for BKV DNA	80	0	80	100%

The IC Ct cut-off value is set at 35 for plasma samples collected in EDTA and urine collected without preservatives when tested with ELITe InGenius, ELITe BeGenius and MyGenius PRO.

The IC Ct cut-off value is set at 35 for whole blood samples collected in EDTA when tested with ELITe InGenius, and ELITe BeGenius.

12.12 Diagnostic Sensitivity: confirmation of positive samples

TThe diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated in association with **ELITe InGenius** or **ELITe BeGenius** analysing clinical samples certified positive for BKV DNA or spiked with reference material. As **ELITe BeGenius** showed equivalent analytical performances to **ELITe InGenius**, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic Sensitivity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius and vice versa.

As **MyGenius PRO** showed equivalent analytical performances to ELITe InGenius in association to urine matrix and plasma matrix, the diagnostic performances of the assay performed on the two instruments and urine and plasma are also considered equivalent. Therefore, the Diagnostic Sensitivity of the assay obtained in association with ELITe InGenius is also applicable to MyGenius PRO.

The results are summed up in the following table.

Table 29 Diagnostic Sensitivity

Samples	N	Positive	Negative	% Diagnostic Sensitivity
Plasma collected in EDTA and positive for BKV DNA	34	34	0	100%
Plasma collected in EDTA and spiked for BKV	24	24	0	
Total	58	58	0	
Urine without preservatives positive for BKV DNA	67	67	0	100%
Whole blood collected in EDTA and positive for BKV DNA	52	52	0	100%
Whole blood collected in EDTA and spiked for BKV	28	28	0	

NOTE

The complete data and results from the tests carried out to evaluate the product's performance characteristics with matrices and instruments are recorded in the Product Technical File for the "BKV ELITe MGB® Kit", FTP175PLD.

13 SPECIMENS AND CONTROLS FOR ABI 7500 Fast Dx Real-Time PCR Instrument

13.1 Specimens

The following specimens and nucleic acid extraction methods are validated for use with the **BKV ELITe MGB Kit** using the ABI 7500 Fast Dx Real-Time PCR Instrument.

Table 30

Specimen type	Kit/Method	Protocol	Input volume (µL)	Elution volume (µL)	Primary tube minimum volume (µL)	Special instruction
Plasma	ELITe GALAXY	xNA Extraction (Universal)	300	200	400-650	Add 10 µL/ sample of CPE to the IC + Carrier solution

13.2 Interfering substances

The DNA extracted from the sample must not contain heparin, haemoglobin, dextran, Ficoll®, ethanol or 2-propanol in order to prevent inhibition problems and the possibility of frequent invalid results.

High quantity of human genomic DNA in the DNA extracted from the sample may inhibit the amplification reaction.

There are no data available concerning inhibition caused by antiviral, antibiotic, chemotherapeutic or immunosuppressant drugs.

Do not use samples collected in heparin, which is a known reverse transcription and PCR inhibitor

13.3 Amplification controls

It is mandatory to validate each amplification session with a Negative Control reaction and a Positive Control reaction.

For the Negative Control, use molecular biology grade water (not provided with this kit) added to the reaction in place of the DNA extracted from the sample.

For the Positive Control, use the **BKV - ELITe Positive Control** product or the **BKV - ELITe Standard** product.

13.4 Quality controls

Verification of the extraction and PCR procedure is recommended. Archived samples or certified reference material may be used. External controls should be used in accordance with local, state, and federal accrediting organizations, as applicable.

14 ABI 7500 Fast Dx Real-Time PCR Instrument PROCEDURE

14.1 Setting of the real time amplification session

(To perform in the amplification / detection of amplification products area)

When **7300 Real-Time PCR System** instrument is used.

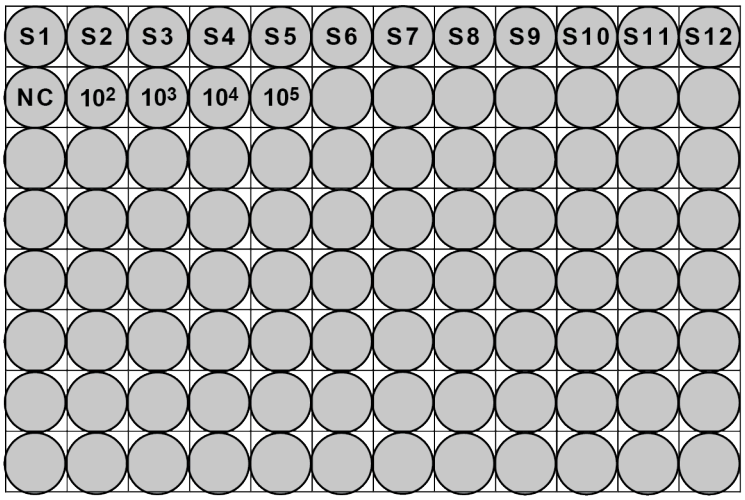
Before starting the session, referring to the instrument documentation, it is necessary to:

- switch on the real time thermal cycler, switch on the computer, run the dedicated software and open an "absolute quantification" session;
- set (Detector Manager) the "detector" for the BKV probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it “BKV”;
- set (Detector Manager) the "detector" for the internal control probe with the "reporter" = "VIC" (AP525 is analogous to VIC) and the "quencher" = "none" (non fluorescent) and call it “IC”;
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference" = "ROX" (AP593 is used instead of ROX, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

NOTE

In order to determine the DNA titre in the starting sample, set up a series of reactions with the **Q -PCR Standards** (10^5 copies, 10^4 copies, 10^3 copies, 10^2 copies) to obtain the **Standard curve.**

See below, by way of example, how to set up the quantitative analysis of 12 samples.



Legend: S1 -S12: Samples to be analysed; **NC**: Negative Control of amplification;

10 ²: 10^2 standard copies; **10³**: 10^3 standard copies; **10 ⁴**: 10^4 standard copies; **10 ⁵**: 10^5 standard copies.

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol > Thermal Profile) the parameters of the **thermal cycle**:

- add to amplification stage the step (Add Step) of **extension at 72°C**;

NOTE

The fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the step of hybridization at 60°C.

- modify timing as indicated in the table **"Thermal cycle"**;
- set the number cycles to **45**;
- set the volume for the software emulation of thermal transfer to reaction ("Sample volume") to **30 µL**;

Table 31 Thermal cycle 7300

Stage	Temperatures	Timing
Decontamination	50 °C	2 min.
Initial denaturation	94 °C	2 min.
Amplification and detection (45 cycles)	94 °C	10 sec.
	60 °C (fluorescence acquisition)	30 sec.
	72 °C	20 sec.
Dissociation (optional)	95 °C	15 sec.
	40 °C	30 sec.
	80 °C	15 sec.

When a **7500 Fast Dx Real-Time PCR Instrument** is used.

Before starting the session, referring to the instrument documentation, it is necessary to:

- switch on the real time thermal cycler, switch on the computer, run the dedicated software and open an "absolute quantification" session and set “Run mode: Fast 7500”;
- set (Detector Manager) the "detector" for the BKV probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it “BKV”;
- set (Detector Manager) the "detector" for the internal control probe with the "reporter" = "VIC" (AP525 is similar to VIC) and the "quencher" = "none" (non fluorescent) and call it “IC”;
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference" = "Cy5" (AP593 is used instead of Cy5, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

NOTE

In order to determine the DNA titre in the starting sample, set up a series of reactions with the Q - PCR Standards (10^5 copies, 10^4 copies, 10^3 copies, 10^2 copies) to obtain the Standard **curve.**

The set up of the quantitative analysis of 12 samples is shown, by way of example, in the previous paragraph describing the procedure for the **7300 Real Time PCR System** instrument.

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol > Thermal Profile) the parameters of the **thermal cycle**:

- add to amplification stage the step (Add Step) of **extension at 72 °C**;

NOTE

The fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the step of hybridization at 60 °C

- modify timing as indicated in the table **"Thermal cycle"**;
- set the number cycles to **45**;
- set the volume for the software emulation of thermal transfer to reaction ("Sample volume") to **30 µL**;
- optional: add the dissociation stage (Add Dissociation Stage) and set the temperature from **40 °C** to **80 °C**

.

Table 32 Thermal cycle 7500

Stage	Temperatures	Timing
Decontamination	50 °C	2 min.
Initial denaturation	94 °C	2 min.
Amplification and detection (45 cycles)	94 °C	10 sec.
	60 °C (fluorescence acquisition)	30 sec.
	72 °C	20 sec.
Dissociation (optional)	95 °C	15 sec.
	40 °C	1 min.
	80 °C	15 sec.
	60 °C	15 sec.

14.2 Real-time PCR session set-up

(Performed by the **ELITe GALAXY** instrument)

To perform the PCR session set up:

- thaw the **Q-PCR Mix** tubes required for the session (each tube is sufficient for **25 reactions**)
- thaw the **Positive Control** (qualitative analysis: detection of extracted DNA) or the **Q - PCR Standard** (quantitative analysis: quantification of extracted DNA) tubes
- mix gently the reagents and spin down the contents for 5 seconds
- prepare the **Negative Control** (not provided) as per the instruction of use of the instrument
- prepare a **Q-PCR microplate**. Handle it with powderless gloves and do not damage the wells

NOTE

To prepare the PCR on the **ELITe GALAXY**, load the elution microplate containing the extracted DNA samples, the reagents and the **Q-PCR microplate** as indicated in the instrument user manual and follow the steps on the GUI.

The instrument automatically performs the PCR set-up dispensing in each well of the **Q-PCR microplate**:

- **20 µL** of **Q-PCR Mix**
- **20 µL** of **extracted DNA** / **Q-PCR Standard** / **Controls**

NOTE

If not all the Q—PCR Mix is used, store the remaining volume in the dark at -20°C for no longer than one month. Freeze and thaw the Q—PCR Mix for a maximum of **5 TIMES**.

After the PCR set-up performed by the instrument:

- seal the **Q-PCR microplate** with an optical seal
- transfer the **Q-PCR microplate** onto the **7500 Fast Dx Real-Time PCR Instrument** and start the PCR. Save the run file with a unique and recognizable name (e.g. "year-month-day-TARGET-EGSpA")

NOTE

At the end of the PCR the **Q-PCR microplate** must be discarded following all governmental and environmental regulations. In order to avoid spilling the PCR products, the **optical seal must not be removed from the Q-PCR microplate**.

14.3 General settings for analysis of results

Before starting the analysis, refer to the instrument documentation to:

- manually adjust the calculation range for the **Baseline** (fluorescence background level) from cycle 6 to cycle 15 (Results > Amplification plot > delta Rn vs Cycle);

NOTE

The FAM fluorescence of the BKV probe in a sample with a high concentration of BKV DNA may begin to increase before cycle 15. In this case, lower the **Baseline** calculation range to the cycle at which the FAM fluorescence of the sample begins to increase (Results > Component).

- manually set the thresholds for the detectors:

set the FAM detector “BKV” **threshold** at **0.2**;

set the VIC detector "IC" **threshold** at **0.1**.

The PCR cycle at which a sample’s fluorescence level reaches the **threshold** value determines the **threshold cycle (Ct)** for that sample.

The instrument software automatically analyses the fluorescence levels in the controls, standards and sample reactions and calculates Ct values.

14.4 Qualitative analysis of results

The BKV **Ct** value of the **Positive Control** is used to validate the PCR. The PCR run is valid when results are as described in the following table:

Table 33

Positive Control reaction Detector FAM " BKV "	Assay result	Amplification / Detection
Ct ≤ 25	POSITIVE	CORRECT

If the result of the **Positive Control** is **Ct > 25** or **Ct Undetermined** for Detector FAM “BKV”**,** the session is not valid and must be repeated starting from the PCR step. This may indicate a contamination which could lead to incorrect and false positives results.

NOTE

When the product is used for the quantification of BKV DNA, the **Q - PCR Standard** reactions were set up instead of the **Positive Control** reaction. In this case, validate the amplification and the detection by referring to the amplification reaction of **Q - PCR Standard 10^5** (**Ct ≤ 25**).

The BKV Ct value of the **Negative Control** is used to validate the PCR. The PCR run is valid when results are as described in the following table:

Table 34

Negative control reaction Detector FAM " BKV "	Assay result	Amplification / Detection
Ct Undetermined	**NEGATIVE**	**CORRECT**

If the result of the **Negative control** amplification reaction is different from **Ct Undetermined** for Detector FAM “BKV”, the session is not valid and must be repeated starting from the PCR step. This may indicate that issues occurred during the amplification step (contamination) which could lead to incorrect results and false positive results.

The **Ct** value of BKV in each sample is used to detect the target DNA while the **Ct** value of the internal control is used to validate the extraction, PCR, and detection.

NOTE

Verify by the amplification plot (Results > Amplification plot > delta Rn vs Cycle) that the **Ct** of each sample was determined by a fast and regular increase in fluorescence and not by peaks or an increase in background signal (irregular or high background).

Possible sample results (Results > Report) are described in the following table:

Table 35

Sample reaction		Sample suitability	Assay sample result	BKV DNA
Detector FAM "BKV"	**Detector VIC "IC"**			
Ct Undetermined	**Ct > 35 or Ct Undetermined**	**unsuitable**	**invalid**	**-**
	Ct ≤ 35	**suitable**	**valid, negative**	**NOT DETECTED**

Table 35 (continued)

Ct Determined	**Ct > 35 or Ct Undetermined**	**suitable**	**valid, positive**	**DETECTED**
	Ct ≤ 35	**suitable**	**valid, positive**	**DETECTED**

A sample result of **Ct Undetermined** for BKV and **Ct > 35** or **Ct Undetermined** for the internal control is invalid and indicates an issue during nucleic acid extraction or PCR (e. g., degradation of sample DNA, loss of DNA during extraction, presence of inhibitors in the DNA, inefficient or absent amplification), which may lead to incorrect results. The sample is not suitable for the analysis and the assay needs to be repeated starting from nucleic acid extraction of a new sample.

A sample result of **Ct Undetermined** for BKV and **Ct ≤ 35** for the internal control is a valid result and indicates that BK DNA was not detected in the sample. The sample may contain no BKV DNA or it contains BKV DNA at a concentration lower than the detection limit of the product (see 15 Performance Characteristics page 46). A sample result of **Ct Determined (Ct ≤ 45)** for BKV and **Ct > 35**, **Ct Undetermined**, or **Ct ≤ 35** for the IC is a valid result and indicates that BKV DNA was detected in the sample

NOTE

In case of Ct Determined for BKV and Ct > 35 or Undetermined for the IC, the PCR efficiency of the IC may have been impacted by competition with the high PCR efficiency of the BKV DNA. In this case the sample is suitable, and the positive result is valid.

NOTE

The results obtained with this assay must be interpreted in combination with all relevant clinical observation and laboratory outcomes.

14.5 Quantitative analysis of the results

After qualitative analysis of results, quantitative analysis of the positive samples can be performed.

In the amplification reactions of the four **Q - PCR standards**, the BKV **Ct** values are used to calculate the **Standard Curve** (Results > Standard Curve) for the amplification session and to validate the amplification and the detection as described in the following table:

Table 36

Standard Curve Detector FAM " BKV "	Acceptability range	Amplification / Detection
Correlation coefficient (R2)	**0.990 ≤ R2 ≤ 1.000**	**CORRECT**

If the **Correlation coefficient (R2)** value does not fall within the limits, the session is not valid and must be repeated starting from the PCR step. This may indicate an issue during the PCR or the detection step (e. g., incorrect dispensation or degradation of the Q-PCR Mix or of the standards, incorrect placement of the standards, incorrect thermal cycle settings or cross-contamination), which may lead to incorrect results.

Table 37

Sample result for detector FAM “BKV”	BKV copies per reaction
Quantity > 1×10^6	**MORE THAN 1×10^6**
1×10^1 ≤ Quantity ≤ 1×10^6	**= Quantity**
Quantity < 1×10^1	**LESS THAN 10**

The results (**Quantity**) of each sample (Results > Report) are used to calculate the copies of BKV present in the specimen used in the extraction (**Nc**) according to this formula:

Table 38

$$Nc = \frac{Ve \times Quantity}{Vc \times Va \times Ep}$$

where:

Ve is the total volume **in µL** of the extracted DNA sample (elution volume)

Quantity is the **copies/reaction** of the sample calculated by the instrument software (PCR result)

Vc is the volume of the specimen used for nucleic acid extraction (input volume) expressed in the required unit of measurement

Va is the volume in **µL** of the extracted DNA sample (eluate) used in the PCR

Ep is the efficiency of the procedure (extraction and PCR) **expressed as a decimal**

To convert the sample quantity from copies/mL to IU/mL, multiply the copies/mL value by the **conversion factor (Fc)**. The Fc was calculated using calibrated certified reference material ("1st WHO International Standard for BK Virus DNA for Nucleic Acid Amplification Techniques", NIBSC) (See 15 Performance Characteristics page 46).

For convenience, the following are simplified formulas in which Ve/(Vc x Va x Ep) and its conversion to IU/mL have been calculated.

Table 39

Matrix	Nucleic acid extraction method	Ve/ (Vc x Va x Ep)	Formula to quantify Nc (copies/mL)	Fc (IU/copies)	Formula to quantify Nc (IU/mL)
plasma	ELITe GALAXY	35	35 x Quantity	4.1	143.5 x Quantity

15 PERFORMANCE CHARACTERISTICS WITH ABI 7500 Fast Dx Real-Time PCR Instrument

15.1 Analytical sensitivity: Limit of detection (LoD)

The Limit of Detection (LoD) of the assay in association to Plasma EDTA was verified on the ELITe GALAXY and ABI 7500 instruments, by testing a panel of BKV negative matrix spiked with reference material of BKV (1st WHO international standard for BKV virus DNA, NIBSC code 14/212, United Kingdom). Probit regression analysis was performed on the results, and the LoD estimated as the concentration corresponding to 95% probability of a positive call.

The result is reported in the following table.

Table 40 Limit of Detection for Plasma samples and ELITe GALAXY

% Positivity	LoD	95% confidence range	
		lower limit	upper limit
95%	**189 copies / mL**	119 copies / mL	462 copies / mL
95%	**779 IU / mL**	490 IU / mL	1,903 IU / mL

The LoD as copies / mL for each matrix is calculated by applying the specific conversion factor reported at paragraph 15.7 Conversion to International Units page 49

15.2 Linear measuring range

The linear measuring range of the assay was determined on **ABI 7500 Fast Dx** using a panel of dilution of a plasmid DNA containing the amplification product.

The linear measuring range as copies / mL is calculated by applying the specific conversion factor reported at paragraph 15.7 Conversion to International Units page 49.

The final results are summarized in the following table.

Table 41 Linear measuring range for Plasma EDTA samples and ABI 7500

Unit of Measure	lower limit	upper limit
IU / mL	41	41,000,000
copies / reaction	10	1,000,000

15.3 Potential interfering markers: Cross-reactivity

The Potential cross-reactivity of unintended organisms that may be found in clinical specimens was evaluated by *in silico* analysis. The analysis showed no significant homology with other unintended organisms (viruses, bacteria, protozoa and fungi). Therefore, no cross-reactivity should be expected.

The absence of cross-reactivity with potential interfering organisms was also verified through the analysis of the panel of unintended organisms (ATCC, NIBSC) at high titre.

The results are reported in the following table.

Table 42

Organism	Pos. / Rep.	Outcome
HSV1	0 / 3	No cross-reactivity
HSV2	0 / 3	No cross-reactivity
CMV	0 / 3	No cross-reactivity
EV	0 / 3	No cross-reactivity
VZV	0 / 3	No cross-reactivity
ADV	0 / 3	No cross-reactivity
EBV	0 / 3	No cross-reactivity
JCV	0 / 3	No cross-reactivity
HHV6	0 / 3	No cross-reactivity

All potentially interfering markers tested showed no cross-reactivity for the BKV target amplification using the BKV ELITe MGB Kit.

15.4 Potential interfering markers: Inhibition

The potential inhibition of unintended organisms that may be found in clinical specimens was evaluated for the assay through the analysis of a panel of unintended organisms in BKV positive samples, from different providers (ATCC, NIBSC).

The results are reported in the following table.

Table 43

Organism	Pos. / Rep.	Outcome
HSV1	3 / 3	No interference
HSV2	3 / 3	No interference
CMV	3 / 3	No interference
EV	3 / 3	No interference
VZV	3 / 3	No interference
ADV	3 / 3	No interference
EBV	3 / 3	No interference
JCV	3 / 3	No interference
HHV6	3 / 3	No interference

All potential interfering organisms tested showed no interference of the BKV target detection and quantification using the BKV ELITe MGB Kit.

15.5 Repeatability

The Repeatability Within-run, between-run and between-day of the assay was evaluated on ABI 7500 by analysis of a panel of samples spiked by plasmid DNA containing the BKV amplification product and one negative sample.

An example of Within-run Repeatability (on one session) results is shown in the tables below.

Table 44 Within —run Repeatability on ABI 7500

Sample copies / reaction	BKV			
	Pos. / Rep.	Mean Ct	SD	% CV
50,000 target + 150,000 IC	12 / 12	23.89	0.15	0.64
5,000 target + 150,000 IC	12 / 12	27.07	0.10	0.37
500 target + 150,000 IC	12 / 12	30.41	0.17	0.56
10 target + 150,000 IC	12 / 12	37.97	0.92	2.43
150,000 IC	0 / 12	-	-	-

An example of Between-run Repeatability (on two sessions) results is shown in the tables below.

Table 45 Between—run Repeatability on ABI 7500

Sample copies / reaction	BKV			
	Pos. / Rep.	Mean Ct	SD	% CV
50,000 target + 150,000 IC	24/ 24	23.91	0.13	0.53
5,000 target + 150,000 IC	24/ 24	27.05	0.11	0.41
500 target + 150,000 IC	24/ 24	30.40	0.20	0.66
10 target + 150,000 IC	24/ 24	36.62	0.70	1.91
150,000 IC	0 / 24	-	-	-

In the Repeatability test, the BKV ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV lower than 5%.

15.6 Reproducibility

The Reproducibility of the assay was evaluated on ABI 7500 by analysis of the results of 10 QC tests of BKV ELITe MGB Kit product.

A summary of the results analysis of Ct value of BKV target and Internal Control amplified using BKV ELITe MGB Kit is shown in the tables below:

Table 46 Reproducibility on ABI 7500

Sample copies / reaction	N	Mean Ct	SD	% CV	Outcome
100,000 target	30	22.70	0.34	1.51	Passed
50,000 target + 150,000 IC	30	23.54	0.33	1.39	Passed
5,000 target + 150,000 IC	30	26.81	0.40	1.49	Passed
500 target + 150,000 IC	30	30.18	0.47	1.54	Passed
10 target + 150,000 IC	90	36.16	0.67	1.86	Passed
150,000 IC	30	22.76	0.25	1.09	Passed
6,000 IC	90	28.11	0.32	1.15	Passed

15.7 Conversion to International Units

The Conversion Factor to International Unit of BKV ELITe MGB Kit and the product component “BKV ELITe Standard” in association with ELITe GALAXY and ABI 7500 Fast Dx Real-Time PCR Instrument, was calculated analyzing a panel of serial dilutions (steps of 0.5 Log) of the “1st WHO International Standard for BK Virus DNA” (NIBSC, UK, code 14/212) in BKV DNA negative plasma collected in EDTA was tested.

The conversion factor was calculated as the anti-log of the mean of Differences (10^{Md}) between the assigned Log Iu / mL value and the measured Log copies / mL value and resulted 4.12 IU / copy for the Plasma collected in EDTA.

15.8 Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative clinical samples, was evaluated analyzing, in association with ELITe GALAXY and ABI 7500 Fast Dx Real-Time PCR Instrument, samples presumably negative for BKV DNA.

The results are summed up in the following table

Table 47 Diagnostic specificity

Sample	N	positive	negative	% Diagnostic Specificity
Plasma collected in EDTA and negative for BKV DNA	52	0	52	100 %

15.9 Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated analyzing, in association with ELITe GALAXY and ABI 7500 Fast Dx Real-Time PCR Instrument, samples certified positive for BKV DNA and spiked samples.

The results are summed up in the following table.

Table 48 Diagnostic sensitivity

Samples	N	positive	negative	% Diagnostic Sensitivity
Plasma collected in EDTA and positive for BKV DNA	9	9	0	100 %
Plasma collected in EDTA and spiked for BKV	42	42	0	
Total	51	51	0	

NOTE

The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instruments are recorded in the Product Technical File "BKV ELITe MGB® Kit", FTP 175PLD.

16 REFERENCES

S. W. Aberle et al. (2002) *J Clin Virology* 25: S79 - S85

C. N. Kotton et al. (2025) Transplantation 109: 1066-1110

E. A. Lukhtanov et al. (2007) *Nucleic Acids Res.* 35: e30

17 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples:

- plasma collected in EDTA (ELITe InGenius, ELITe BeGenius, MyGenius PRO and ABI 7500 Fast Dx Real-Time PCR Instrument only)
- urine (ELITe InGenius, ELITe BeGenius and MyGenius PRO only)
- whole blood collected in EDTA (ELITe InGenius and ELITe BeGenius only)

Currently there are no data available concerning product performance with other clinical samples such as: suspensions of leukocytes, suspensions of granulocytes.

Plasma collected in EDTA shall be obtained from whole blood stored at room temperature or +2 / +8 °C for no longer than 24 hours.

Do not use DNA extracted from heparinized samples with this product: heparin inhibits the amplification reaction of nucleic acids and causes invalid results.

Do not use extracted DNA that is contaminated with haemoglobin, dextran, Ficoll®, ethanol or 2-propanol with this product: these substances inhibit the amplification reaction of nucleic acids and may cause invalid results.

Do not use with this product extracted DNA containing high quantity of human genomic DNA that may inhibit the amplification reaction of nucleic acids.

There are no data available concerning inhibition caused by antiviral, antibiotic, chemotherapeutic, or immunosuppressant drugs.

The results obtained with this product depend on proper identification, collection, transport storage and processing of the samples. To avoid incorrect results, it is therefore necessary to take care during these steps and to carefully follow the instructions for use provided with the product.

Due to its high analytical sensitivity, the Real Time PCR method is susceptible to contamination from positive samples, positive controls, and PCR products. Such cross-contamination may cause false positive results. The product format is designed to reduce this, but only good laboratory practices and compliance with these Instructions for Use can prevent it.

This product must be handled by qualified personnel trained in the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other people.

This product requires the use of personal protective equipment and areas that are suitable for the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other people.

This product requires the use of personal protective equipment and instruments dedicated to work session setup to avoid false positive results.

To avoid incorrect results, this product must be handled by professional personnel, qualified and trained in molecular biology techniques such as extraction, PCR and detection of nucleic acids.

Due to inherent differences between technologies, it is recommended that users perform method correlation studies to estimate technology differences prior to switching to a new technology.

A negative result obtained with this product indicates that the target DNA is not detected in the DNA extracted from the sample; however, it cannot be excluded that the target DNA has a lower titer than the product detection limit (see 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius, ELITe BeGenius and MyGenius PRO page 29). In this case the result could be a false negative.

Results obtained with this product may sometimes be invalid due to failure of internal control. In this case the sample shall be retested, starting from extraction, which can lead to a delay in obtaining final results.

Possible polymorphisms, insertions or deletions within the region of the DNA targeted by the product primers and probes may impair detection and quantification of target DNA.

As with any other diagnostic medical device, the results obtained with this product must be interpreted in combination with all relevant clinical observations and laboratory results.

As with any other diagnostic medical device, there is a residual risk of obtaining invalid or erroneous results with this product. This residual risk cannot be eliminated or further reduced. In some cases, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient. However, this residual risk associated to the intended use of the product has been weighed against the potential benefits to the patient and it has been assessed acceptable.

18 TROUBLESHOOTING

ELITe InGenius and ELITE BeGenius

Table 49

Invalid Q-PCR Standard reaction, Standard curve or Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of Q-PCR Mix, Q-PCR Standards and Positive Control. Check the volumes of Q-PCR Mix, Q-PCR Standards and Positive Control.
PCR Mix degradation.	Do not use the Q-PCR Mix for more than 5 independent sessions (3 hours each in the Inventory Area, Cool Block or in the Cooler Unit). Do not use the Q-PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit) Do not leave the Q-PCR Mix at room temperature for more than 30 minutes. Use a new tube of Q-PCR Mix.
Q-PCR Standards or Positive Control degradation.	Do not use the Q-PCR Standard for more than 4 independent sessions (2 hours each in the Extraction Area or in the Cooler Unit). Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Extraction Area or in the Cooler Unit). Use new tubes of Q-PCR Standards or Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

Table 50

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of Q-PCR Mix and Negative Control. Check the volumes of Q-PCR Mix and Negative Control.
Contamination of the Negative Control.	Do not use the Negative Control for more than 1 session. Use a new aliquot of molecular biology grade water.
Contamination of the PCR Mix.	Use a new tube of Q-PCR Mix.
Contamination of the extraction area, Racks, Inventory Block or Cooler Unit.	Clean surfaces with aqueous detergents, wash lab coats, replace tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Table 51

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of Q-PCR Mix, Internal Control, and sample. Check the volumes of Q-PCR Mix, Internal Control and sample.
PCR Mix degradation.	Do not use the Q-PCR Mix for more than 5 independent sessions (3 hours each in the Inventory Area or in the Cooler Unit). Do not use the Q-PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the Q-PCR Mix at room temperature for more than 30 minutes. Use a new tube of Q-PCR Mix.
Internal Control template degradation.	Use a new tube of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification of eluted sample as is or with a 1:2 dilution in molecular biology grade water in a “PCR Only” session. Repeat the extraction of the sample with a 1:2 dilution in molecular biology grade water in an “Extract + PCR” session. For BAL/BA samples, perform or repeat the liquefaction of the primary sample as reported in 8 SPECIMENS AND CONTROLS for ELITe InGenius, ELITe BeGenius AND MyGenius PRO page 9
Instrument error.	Contact ELITechGroup Technical Service.

Table 52

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but Tm different from that of the other samples and that of the Standards or Positive Control.	Check for target Ct lower than 30. High quantity of amplification product at the end of the reaction may interfere with the melting curve analysis. Repeat the sample amplification to confirm the presence of target with a possible mutation. The target in the sample should be sequenced to confirm mutation.

Table 53

Error in Ct calculation	
Possible Causes	**Solutions**
Too high concentration of target in the sample or sample with anomalous fluorescence signal.	If significant amplification is observed in PCR plot, select the track related to the sample and manually approve the result as positive. If no amplification is observed in PCR plot select the track related to the sample and manually approve the result as negative or leave it as invalid. If a Ct value is required: - repeat the amplification of eluted sample with a 1:10 dilution in molecular biology grade water in a “PCR Only” session - repeat the extraction of the sample with a 1:10 dilution in molecular biology grade water in an “Extract + PCR” session.

Table 54

Abnormal high rate of positive results within the same session (reactions with similar late Ct values)	
Possible Causes	**Solutions**
Sample-to-sample contamination during preanalytical steps.	Clean the micropipette with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner after pipetting each sample. Do not use Pasteur pipettes. The pipettes must be of the positive displacement type or used with aerosol filter tips. Introduce samples in the last positions of the instruments, as indicated by the GUI. Follow the loading sequence indicated by the software.
Laboratory environmental contamination.	Clean all surfaces in contact with the operator and samples (including the pipettes) with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner. Perform an U.V. decontamination cycle. Use a new tube of Q-PCR Mix and / or Internal Control

MyGenius PRO

Table 55

Invalid Q-PCR Standard reaction, Standard curve or Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the volumes of Q-PCR Mix, Q-PCR Standards and Positive Control. It is important that the tubes used on MyGenius PRO have not been previously used by other platforms or for setting sessions on open platforms because the instrument reads the label and automatically assigns the maximum number of replicates that can be run if that tube has never been loaded on MyGenius PRO. Therefore, if a non-new tube is used, the volume may not be sufficient.
PCR Mix degradation.	Do not use the Q-PCR Mix for more than 7 hours in the Carousel or for more than 3 hours in the Carousel for five times. Do not leave the Q-PCR Mix at room temperature for more than 30 minutes. Use a new tube of Q-PCR Mix.
Q-PCR Standards or Positive Control degradation.	Do not use the Q-PCR Standard for more than 4 independent sessions (2 hours each in the Carousel). Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Carousel). Use new tubes of Q-PCR Standards or Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

Table 56

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the volumes of Q-PCR Mix and Negative Control. It is important that the tubes used on MyGenius PRO have not been previously used by other platforms or for setting sessions on open platforms because the instrument reads the label and automatically assigns the maximum number of replicates that can be run if that tube has never been loaded on MyGenius PRO. Therefore, if a non-new tube is used, the volume may not be sufficient.
Contamination of the Negative Control.	Do not use the Negative Control for more than 1 session and for more than 8 hours. Use a new tube of Negative Control.
Contamination of the PCR Mix.	Use a new tube of Q-PCR Mix.
Contamination of the extraction area, Racks, Inventory Block or Cooler Unit.	Clean surfaces with aqueous detergents, wash lab coats, replace tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Table 57

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the volumes of Q-PCR Mix, Internal Control and Samples. It is important that the tubes used on MyGenius PRO have not been previously used by other platforms or for setting sessions on open platforms because the instrument reads the label and automatically assigns the maximum number of replicates that can be run if that tube has never been loaded on MyGenius PRO. Therefore, if a non-new tube is used, the volume may not be sufficient.
PCR Mix degradation.	Do not use the Q-PCR Mix for more than 7 hours in the Carousel or for more than 3 hours in the Carousel for five times. Do not leave the Q-PCR Mix at room temperature for more than 30 minutes. Use a new tube of Q-PCR Mix.
Internal Control template degradation.	Do not use the Internal Control for more than 8 hours. Use a new tube of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the extraction and amplification of the sample. Repeat the extraction and amplification of the sample with a 1:2 dilution in molecular biology grade water.
Too high concentration of target in the sample or sample with anomalous fluorescence signal.	If a Ct value is required, repeat the extraction and amplification of the sample with a 1:10 dilution in molecular biology water.
Instrument error.	Contact ELITechGroup Technical Service.

Table 58

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but Tm different from that of the other samples and that of the Standards or Positive Control.	Check for target Ct lower than 30. High quantity of amplification product at the end of the reaction may interfere with the melting curve analysis. Repeat the sample extraction and amplification to confirm the presence of target with a possible mutation. The target in the sample should be sequenced to confirm mutation

Table 59

Error in Ct calculation	
Possible causes	**Solutions**
Too high concentration of target in the sample or sample with anomalous fluorescence signal.	If a significant amplification appears in the PCR plot, the result should be considered positive. If no amplification appears in the PCR plot, the result should be considered negative or invalid. If a Ct value is required, repeat the extraction and amplification of the sample with a 1:10 dilution in molecular biology water.

Table 60

Abnormal high rate of positive results within the same session (reactions with similar late Ct values)	
Possible Causes	**Solutions**
Sample-to-sample contamination during preanalytical steps.	Clean the micropipette with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner after pipetting each sample. Do not use Pasteur pipettes. The pipettes must be of the positive displacement type or used with aerosol filter tips. Introduce samples as the last of the day.
Laboratory environmental contamination.	Clean all surfaces in contact with the operator and samples (including the pipettes) with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner. Perform an U.V. decontamination cycle. Use a new tube of Q-PCR Mix and / or Internal Control

Open Platform

Table 61

Invalid Q-PCR Standard reaction, Standard curve or Positive Control reaction	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Check the volumes of PCR Mix, Q-PCR Standards and Positive Control dispensed in the Q-PCR microplate.
Q-PCR Mix degradation.	Do not freeze and thaw the PCR mix more than 5 times. Do not leave the Q-PCR Mix at room temperature for more than 30 minutes. Use a new tube of Q-PCR Mix.

Table 61 (continued)

Invalid Q-PCR Standard reaction, Standard curve or Positive Control reaction	
Possible Causes	**Solutions**
Q-PCR Standards or Positive Control degradation.	Do not freeze and thaw the Q-PCR standard more than 4 times. Use new tubes of Q-PCR Standards or Positive Control.
Instrument setting error.	Check the position of PCR Mix, Q-PCR Standards and Positive Control on the instrument. Check the thermal cycle settings on the instrument.

Table 62

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of Q-PCR Mix and Negative Control. Check the volumes of Q-PCR Mix and Negative Control.
Microplate badly sealed.	Take care when sealing the Q-PCR microplate with the optical seal.
Contamination of the Negative Control.	Do not use the Negative Control for more than 1 session. Use a new aliquot of molecular biology grade water.
Contamination of the PCR Mix.	Use a new tube of Q-PCR Mix.
Contamination of the preparation area, racks and micropipette.	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use.

Table 63

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of Q-PCR Mix, Internal Control and sample. Check the volumes of Q-PCR Mix, Internal Control and sample.
PCR Mix degradation.	Do not freeze and thaw the PCR mix more than five times. Do not leave the Q-PCR Mix at room temperature for more than 30 minutes. Use a new tube of Q-PCR Mix
Internal Control template degradation.	Use a new tube of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification of eluted sample with a 1:2 dilution in molecular biology grade water. Repeat the extraction of the sample with a 1:2 dilution in molecular biology grade water.

Table 64

Irregular or high background fluorescence in the reactions	
Possible causes	**Solutions**
Incorrect dispensing of sample.	Check the volumes of reagents and samples dispensed in the Q-PCR microplate.
Baseline setting error.	If the calculation range for the Baseline set from cycle 6 to cycle 15 is not proper to normalize the background, set the calculation range within cycles where the background fluorescence has already stabilized (check Results > Component) and the target fluorescence has not yet started to increase.

Table 65

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but different from that of the other samples and that of the Standards or Positive Control.	Check for target Ct lower than 30. High quantity of amplification product at the end of the reaction may interfere with the melting curve analysis. Repeat the sample amplification to confirm the presence of target with a possible mutation. The target in the sample should be sequenced to confirm mutation.

19 SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.
	Use by (last day of month).
IVD	*in vitro* diagnostic medical device.
CE 0123	Fulfilling the requirements of the IVDR Regulation 2017/746/EC for *in vitro* diagnostic medical device. Certification released by TÜV SÜD Product Service GmbH, Germany.
UDI	Unique Device Identification
	Contains sufficient for "N" tests.
	Consult instructions for use.
CONT	Contents.
	Keep away from sunlight.
	Manufacturer.

20 NOTICE TO THE USERS

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and /or the patient is established. To inform ELITechGroup S. p. A., manufacturer of this device, please use the following mail address: egspa.vigilance@elitechgroup.com.

A “Summary of Safety and Performance” will be made available to the public via the European database on medical devices (Eudamed) when this informatic system will be functional. Before the notice of full functionality of Eudamed has been published, the “Summary of Safety and Performance” will be made available to the public upon request by email at emd.support@elitechgroup.com, without undue delay.

21 NOTICE TO PURCHASER: LIMITED LICENSE

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ELITe MGB® detection reagents and ELITech platforms (ELITe InGenius®, ELITe BeGenius®, MyGenius PRO®) are protected by granted patents and pending applications.

Any use of the reagents or related data outside the scope of this kit requires prior written authorization from ELITechGroup S.p.A.

ELITe MGB ® detection reagents are covered by one or more of U. S. Patent numbers 7319022, 7348146, 7541454, 7671218, 7723038, 7767834, 8163910, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

ELITe InGenius®, ELITe BeGenius®and MyGenius PRO® technologies are covered by patents and pending applications.

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Appendix A BKV ELITe MGB Kit used in association with Genius series® platforms



CAUTION

This document is a simplified version of the official instruction for use. Please refer to the complete document before use: www.elitechgroup.com

Intended use

The product **BKV ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as quantitative nucleic acids Real-Time PCR assay for the detection and quantification of the **DNA of human Polyomavirus BK (BKV)** extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of plasma collected in EDTA, urine collected without preservatives and whole blood collected in EDTA.

The assay is also validated in association with the **MyGenius PRO®** (registration name ELIVERSE®) instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of plasma collected in EDTA and urine collected without preservatives.

The assay is also validated in association with the **ELITe GALAXY**, automatic extraction and PCR set-up system and **7500 Fast Dx Real-Time PCR Instrument**, Real-Time PCR platform, using human specimens of plasma collected in EDTA.

The product is intended for use as an aid in the diagnosis and monitoring of BKV infections in patients suspected of having or undergoing monitoring of BKV infections.

The results must be interpreted in combination with all relevant clinical observations and laboratory outcomes.

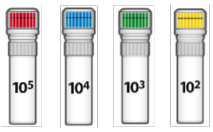
Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target	Large T antigen gene	FAM	BKV
Internal Control	Beta-globin	AP525	IC

Validated matrix

- **Plasma** collected in EDTA
- **Urine** without preservatives
- **Whole blood** collected in EDTA

Kit content and related products

BKV ELITe MGB Kit	BKV ELITe Standard	BKV- ELITe Positive Control
 X 4	 X 2	 X 2
Ready-to-use PCR Mix 4 tubes of 540 µL 96 reactions per kit 5 freeze-thaw cycles per tube, used On Board	Ready-to-use 4 levels: 10^5, 10^4, 10^3, 10^2 2 set of 4 tubes of 200 µL 8 reactions per kit 4 freeze-thaw cycles per tube, used On Board	Ready-to-use PC 2 tubes of 160 µL 8 reactions per kit 4 freeze-thaw cycles per tube, used On Board

Maximum shelf-life: **24 months**

Storage Temperature: **-20 °C**

Other products required not provided in the kit

• ELITe InGenius instrument: INT030. • ELITe BeGenius instrument: INT040. • ELITe InGenius SP 200: INT032SP200.	• CPE - Internal Control: CTRCPE **ELITe InGenius** and **ELITe BeGenius** Consumables (see ELITe InGenius and ELITe BeGenius Instruction for Use)

ELITe InGenius and ELITe BeGenius protocol

› Sample volume › CPE volume › Total elution volume	200 µL(InGenius and BeGenius) 10 µL 100 µL	› Eluate PCR input volume › Q—PCR Mix volume › Frequency of controls › Frequency of calibration	20 µL 20 µL 15 days 60 days

ELITe InGenius and ELITe BeGenius Performances

Matrix	Limit of Detection		Diagnostic Specificity	Diagnostic Sensitivity	Linearity(IU/mL)		Conversion factor (IU / copies)
	IU/mL	copies/mL			IU/mL	copies/mL	
Plasma	215 IU / mL	165 copies / mL	96.2 %	100 %	215→ 13,0 x 10^7	165→ 10,0 x 10^7	1.3
Urine	142 IU / mL	89 copies / mL	100 %	100 %	142→ 16,0 x 10^7	89→ 10,0 x 10^7	1.6
Whole blood	538 IU / mL	117 copies / mL	100 %	100 %	538→ 46,0 x 10^7	117→ 10,0 x 10^7	4.6

Sample preparation

This product is intended for use on the **ELITe InGenius** and **ELITe BeGenius** with the following clinical specimens identified according to laboratory guidelines, and collected, transported, and stored under the following conditions.

Sample type	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	+2 / +8 °C	-20 ± 10 °C	-70 ± 15 °C
Plasma	EDTA	≤ 1 d	≤ 3 d	≤ 30 d	≤ 30 d
Urine	Without preservatives	≤ 4 hours	≤ 1 d	≤ 30 d	≤ 30 d
Whole blood	EDTA	≤ 1 d	≤ 3 d	≤ 30 d	≤ 30 d

EDTA, Ethylenediaminetetraacetic acid; d, day.

ELITe InGenius Procedures

The user is guided step-by-step by the Graphic User Interface (GUI) of ELITe InGenius software to setup the run. All the steps: extraction, Real-Time PCR and result interpretation are automatically performed. Two operational modes are available: complete run (Extract + PCR) or PCR Only.

Before analysis

1. Switch on ELITe InGenius. Log in with username and password. Select the mode “Closed”.	**2.** Verify calibrators: **Q-PCR Standard** in the “Calibration” menu. Verify controls: **Positive Control** and **Negative Control** in the “Controls” menu. Note: All must have been run, approved and not expired.	**3.** Thaw the **PCR Mix** and the **CTRCPE** tubes. Vortex gently. Spin down 5 sec.

Procedure 1 - Complete run: Extract + PCR (e.g., samples)

1. Select “Perform Run” on the touch screen and then click on the run mode «Extract + PCR»	**2.** Insert the Sample Rack with the barcoded samples in the Cooler Unit. The barcode scan is already active	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “100 µL”
4. Select the “Assay protocol” of interest BKV ELITe_PL_200_100 or BKV ELITe_U_200_100 or BKV ELITe_WB_200_100 **Note:** If a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit	**6.** Load the PCR Mix and the Internal Control in the Reagent/Elution Rack and insert it in the Cooler Unit
7. Load “PCR Rack” with “PCR Cassette” and the “Extraction Rack” with the “ELITe InGenius SP 200” extraction cartridges and the required extraction consumables	**8.** Close the door. Start the run	**9.** View, approve and store the results

NOTE

If an Extract Only mode is needed, refer to the instrument user’s manual for procedure.

Procedure 2: PCR Only (e.g., eluates, standards, controls)

1. Select “Perform Run” on the touch screen	**2.** Verify the extraction volumes: Input: “200 µL”, elution: “100 µL”	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the “Assay protocol” of interest: BKV ELITe_PC and BKV ELITe_NC, or BKV ELITe_STD, or BKV ELITe_PL_200_100, BKV ELITe_U_200_100 or BKV ELITe_WB_200_100	**5.** Select the method “PCR Only” and the sample position “Elution Tube”	**6.** Load the PCR Mix in the Inventory Block
7. Load: PCR Cassette rack and the Elution tube rack with the extracted nucleic acid	**8.** Close the door. Start the run	**9.** View, approve and store the results

ELITe BeGenius Procedures

The user is guided step-by-step by the Graphic User Interface (GUI) of ELITe BeGenius software to setup the run. All the steps, extraction, Real-Time PCR and result interpretation, are automatically performed. Two operational modes are available: complete run (Extract + PCR) or PCR Only.

Before analysis

1. Switch on ELITe BeGenius. Log in with username and password. Select the mode “Closed”.	**2.** Verify calibrators: **Q-PCR Standard** in the “Calibration” menu. Verify controls: **Positive Control** and **Negative Control** in the “Controls” menu. Note: All must have been run, approved and not expired.	**3.** Thaw the **PCR Mix** and the **CTRCPE** tubes. Vortex gently. Spin down 5 sec.

Procedure 1 - Complete run: Extract + PCR (e.g., samples)

1. Select “Perform Run” on the touch screen and then click on the run mode «Extract + PCR»	**2.** Insert the Sample Rack with the barcoded samples in the Cooler Unit. The barcode scan is already active	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “100 µL”
4. Select the “Assay protocol” of interest BKV ELITe_Be_PL_200_100 or BKV ELITe_Be_U_200_100 or BKV ELITe_WB_200_100 **Note:** If a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit	**6.** Load the PCR Mix and the Internal Control in the Reagent/Elution Rack and insert it in the Cooler Unit
7. Load “PCR Rack” with “PCR Cassette” and the “Extraction Rack” with the “ELITe InGenius SP 200” extraction cartridges and the required extraction consumables	**8.** Close the door. Start the run	**9.** View, approve and store the results

NOTE
If an Extract Only mode is needed, refer to the instrument user’s manual for procedure.

Procedure 2: PCR Only (e.g., eluates, standards, controls)

1. Select “Perform Run” on the touch screen	**2.** Load the extracted nucleic acid or controls/calibrators barcoded tubes in the Elution Rack and insert it in the Cooler Unit.	**3.**For Standards and Controls: for each “Position”enter the “Reagent name” and the “S/N” (serial number), the “Lot No.” (lot number), the “Exp.Date” (expiry date) and the “T/R” (number of reactions). For eluates: for each “Position” enter the “Sample ID”, the “Sample matrix”, the “Extraction kit” and the “Extracted eluate vol.” (eluate volume).
4. Select the “Assay protocol” of interest: BKV ELITe_PC and BKV ELITe_NC, or BKV ELITe_STD or BKV ELITe_PL_200_100 or BKV ELITe_U_200_100.	**5.** Load the PCR Mix in Reagent / Elution Rack and insert it in the Cooler Unit Load filter tips and the PCR Rack with “PCR Cassette”.	**6.** Close the door. Start the run.
7. View, approve and store the results.		

Appendix B BKV ELITe MGB Kit used in association with MyGenius PRO



CAUTION

This document is a simplified version of the official instruction for use. Please refer to the complete document before use: www.elitechgroup.com

Intended use

The product **BKV ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as quantitative nucleic acids Real-Time PCR assay for the detection and quantification of the **DNA of human Polyomavirus BK (BKV)** extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of plasma collected in EDTA, urine collected without preservatives and whole blood collected in EDTA.

The assay is also validated in association with the **MyGenius PRO®** (registration name ELIVERSE®) instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of plasma collected in EDTA and urine collected without preservatives.

The assay is also validated in association with the **ELITe GALAXY**, automatic extraction and PCR set-up system and **7500 Fast Dx Real-Time PCR Instrument**, Real-Time PCR platform, using human specimens of plasma collected in EDTA.

The product is intended for use as an aid in the diagnosis and monitoring of BKV infections in patients suspected of having or undergoing monitoring of BKV infections.

The results must be interpreted in combination with all relevant clinical observations and laboratory outcomes.

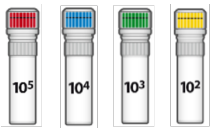
Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target	Large T antigen gene	FAM	BKV
Internal Control	Beta-globin	AP525	IC

Validated matrix

- **Plasma** collected in EDTA
- **Urine** without preservatives

Kit content and related products

BKV ELITe MGB Kit	BKV ELITe Standard	BKV- ELITe Positive Control
 X 4	 X 2	 X 2
Ready-to-use PCR Mix 4 tubes of 540 µL 96 reactions per kit 5 freeze-thaw cycles per tube, used On Board	Ready-to-use 4 levels: 10^5, 10^4, 10^3, 10^2 2 set of 4 tubes of 200 µL 8 reactions per kit 4 freeze-thaw cycles per tube, used On Board	Ready-to-use PC 2 tubes of 160 µL 8 reactions per kit 4 freeze-thaw cycles per tube, used On Board

Maximum shelf-life: **24 months**

Storage Temperature: **-20 °C**

Other products required not provided in the kit

- **MyGenius PRO** (EG SpA ref: INT050)
- **MyGenius PRO Software** version BB-04 (or later)
- **Extraction Cartridge** (EG SpA ref: INT052SP)
- **Negative Control** (EG SpA, ref. CTRNEG)
- **Internal Control Maxi** (EG SpA, ref. ICMAXI)
- **MyGenius PRO** Consumables (see MyGenius PRO Instruction for use)

MyGenius PRO protocol

› Sample volume	200 µL	› Eluate PCR input volume	20 µL
› IC volume	10 µL	› Q—PCR Mix volume	20 µL
› Total elution volume	100 µL	› Frequency of controls	15 days
		› Frequency of calibration	60 days

MyGenius^{PRO} Performances

Matrix	Limit of Detection		Diagnostic Sensitivity	Diagnostic Specificity	Linearity(IU/mL)		Conversion factor (IU / copies)
	IU/mL	copies/mL			IU/mL	copies/mL	
urine	142	89	100%	100%	142→ 16,0 x 10^7	89→ 10,0 x 10^7	1.6
Plasma	215 IU / mL	165 copies / mL	96.2 %	100 %	215→ 13,0 x 10^7	165→ 10,0 x 10^7	1.3

Sample preparation

This product is intended for use on the **MyGenius^{PRO}** with the following clinical specimens identified according to laboratory guidelines, and collected, transported, and stored under the following conditions.

Sample type	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	+2 / +8 °C	-20 ± 10 °C	-70 ± 15 °C
Urine	without preservatives	≤ 4 hours	≤ 1 d	≤ 30 d	≤ 30 d
Plasma	EDTA	≤ 1 d	≤ 3 d	≤ 30 d	≤ 30 d

d, day.

MyGenius PRO Procedures

The user is guided step-by-step by the Graphic User Interface (GUI) of MyGenius PRO software to setup the run. All the steps: extraction, Real-Time PCR and result interpretation are automatically performed. Two operational modes are available: complete run (Extract + PCR) or PCR Only (only for Calibrators and Controls).

1. Switch on **MyGenius PRO**. Log in in **STAND-BY** mode with username and password.	**2.** Load all consumables in the drawers and empty liquid waste tank and solid waste boxes if needed. Press Start button to perform Preparation: after preparation, the instrument will go in Operation mode.	**3.** Verify calibrators: **Q-PCR Standard** in the “Calibration” menu. Verify controls: **Positive Control** and **Negative Control** in the “Controls” menu Note: All must have been run, approved and not expired.

4. Thaw the **PCR Mix** and the **IC MAXI** tubes. Vortex gently. Spin down 5 sec	**5.** Load PCR mixes tubes in the reagent carousel by following GUI indications.	**6.**Load IC MAXI tube(s) in dedicated blue rack and load it in the Auto Sampler area.
7a. If the instrument is connected to the LIS, insert samples in Auto Sampler area using dedicated racks depending on tube diameters used. The extraction automatically will start. **7b**. If the instrument is not connected to the LIS, in Sample list, press “Assign test”, read the barcode of the samples with the external barcode reader, select urine matrix and assign the Assay protocol “BKV ELITe_My_U_IU_200_100” or “BKV ELITe_My_U_cmL_200_100” insert samples in Auto Sampler area using dedicated racks depending on tube diameters used. The extraction automatically will start.		

Appendix C BKV ELITe MGB Kit used in association with ABI 7500 Instrument

CAUTION

This document is a simplified version of the official instruction for use. Please refer to the complete document before use: www.elitechgroup.com

Intended use

The product **BKV ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as quantitative nucleic acids Real-Time PCR assay for the detection and quantification of the **DNA of human Polyomavirus BK (BKV)** extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of plasma collected in EDTA, urine collected without preservatives and whole blood collected in EDTA.

The assay is also validated in association with the **MyGenius PRO®** (registration name ELIVERSE®) instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of plasma collected in EDTA and urine collected without preservatives.

The assay is also validated in association with the **ELITe GALAXY**, automatic extraction and PCR set-up system and **7500 Fast Dx Real-Time PCR Instrument**, Real-Time PCR platform, using human specimens of plasma collected in EDTA.

The product is intended for use as an aid in the diagnosis and monitoring of BKV infections in patients suspected of having or undergoing monitoring of BKV infections.

The results must be interpreted in combination with all relevant clinical observations and laboratory outcomes.

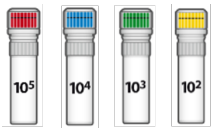
Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target	Large T antigen gene	FAM	BKV
Internal Control	Beta-globin	AP525	IC

Validated matrices

- **Plasma** collected in EDTA

Kit content and related products

BKV ELITe MGB Kit	BKV ELITe Standard	BKV- ELITe Positive Control
 X 4	 X 2	 X 2
Ready-to-use PCR Mix 4 tubes of 540 µL 100 reactions per kit 5 freeze-thaw cycles per tube	Ready-to-use 4 levels: 10^5, 10^4, 10^3, 10^2 2 set of 4 tubes of 200 µL 16 reactions per kit 8 freeze-thaw cycles per tube	Ready-to-use PC 2 tubes of 160 µL 12 reactions per kit 8 freeze-thaw cycles per tube

Maximum shelf-life: **24 months**

Storage Temperature: **-20 °C**

Other products required not provided in the kit

• **ELITe GALAXY:** INT020 • **ELITe GALAXY 300 extraction kit:** INT021EX • **ABI 7500 Fast Dx Real—Time PCR Instrument**	• CPE - Internal Control: CTRCPE • Molecular biology grade water

7500 Real-Time PCR Instrument Performances

Matrix	Limit of Detection	Diagnostic Specificity	Diagnostic Sensitivity	Linearity(IU/mL),	Conversion factor IU/mL to copies/mL	Conversion factor copies/mL to IU/ mL
Plasma	779 IU /mL	100%	100%	41→ $4.1*10^7$	4.1	143.5 x Quantity

7500 Real-Time PCR Instrument Procedures

The procedure below summarized the main steps of the sample analysis with conventional PCR workflow: validated extraction systems, PCR instrument settings, PCR set-up and result interpretation.

Extraction - Validated systems

Extraction	Validated matrix	Sample volume processed	Min. sample volume	Total eluate volume	CPE Internal Control volume
ELITe Galaxy	Plasma	300 µL	400 µL	200 µL	10 µL

Amplification - Settings of 7500 Fast Dx

1. Switch on the thermal-cycler
2. Set “BKV” detector with “FAM” and quencher “none”
3. Set “Internal Control” detector with “VIC” and quencher “none”
4. Set passive fluorescence as “Cy5”
5. Set up the thermal profile as indicated. Fluorescence acquisition must be set during hybridization step at 60° C

Stage	Temperature	Timing
Decontamination	50°C	2 min
Denaturation	94°C	2 min
Amplification	94°C	10 sec
Detection	60°C	30 sec
45 cycles	72°C	20 sec

The melt curve analysis is optional, refer to the complete IFU

Amplification - PCR Set-up

To perform the PCR session set up:

1. Thaw the Q PCR-Mix and Positive Control / Q-PCR standard tubes
2. Mix gently and spin-down
3. prepare the **Negative Control** (not provided)

4. prepare a **Q-PCR microplate**
5. The instrument automatically performs the PCR set-up dispensing in each well of the **Q-PCR microplate 20 µL** of **PCR Mix** and **20 µL** of **extracted DNA** / **Q-PCR Standard** / **Controls**

After the PCR set-up performed by the instrument:

1. seal the **Q-PCR microplate** with an optical seal
2. transfer the **Q-PCR microplate** onto the **7500 Fast Dx Real-Time PCR Instrument** and start the PCR. Save the run file with a unique and recognizable name (e.g. "year-month-day-TARGET-EGSpA")

Amplification - Threshold for qualitative analysis

Instrument	BKV FAM	Internal Control VIC
7500 Fast Dx Real Time PCR	0.2	0.1

Interpretation

Qualitative results

BKV Ct value	Internal Control Ct value	Interpretation
Determined	–	Positive
Undetermined	Ct ≤ 35	Negative
	Ct >35 or Undetermined	Invalid

Quantitative results

The BKV Ct value obtained for each sample and the standard curve generated are used to calculate the quantity of target DNA in the reaction
The sample quantification ranges from approximately 10 to 10^6 copies / reaction or approximately from 41 to 4.1 x 10^7 IU/mL

ELITechGroup S.p.A.
C.so Svizzera, 185, 10149 Torino ITALY
Tel. +39-011 976 191
E. mail: emd.support@elitechgroup.com
WEB site: www.elitechgroup.com